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2. Patent application number
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3. Full name, address and postcode of the or of each applicant *(underline all surnames)*
K.U.Leuven Research and Development - Groot Begijnhof 59 - 3000 Leuven

Represented by Dr. Patrick Chaltin, T.T.O.

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation
Belgium

07665649003

4. Title of the invention
New phosponate nucleosides

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*
K.U.Leuven R&D
care off:
Hubert Velge
Neaves Cottage
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East Sussex BN8 5UA

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Number of earlier application	Date of filing *(day / month / year)*

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11. I/We request the grant of a patent on the basis of this application.

Dr. Patrick Chaltin

Signature [signature] Date 04/03/04

12. Name and daytime telephone number of person to contact in the United Kingdom: Hubert Velge +44 7940 540 397

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NEW PHOSPONATE NUCLEOSIDES

FIELD OF THE INVENTION

The present invention relates to a series of novel phosponate nucleosides, more specifically comprising a phosphonalkoxy substituted five-membered, saturated or unsaturated, oxygen, nitrogen or sulfur containing ring coupled to a pyrimidine or purine base. The invention further relates to compounds having HIV (Human Immunodeficiency Virus) replication inhibiting properties and to compounds having antiviral activities with respect to other viruses. The invention also relates to methods for preparation of all such compounds and pharmaceutical compositions comprising them. The invention further relates to the use of said compounds as a medicine and in the manufacture of a medicament useful for the treatment of subjects suffering from HIV infection, as well as for treatment of other viral, retroviral or lentiviral infections and to the treatment of animals suffering from FIV, viral, retroviral or lentiviral infections. The invention also provides methods of treatment or prevention of a viral infection in a mammal.

BACKGROUND OF THE INVENTION

A retrovirus designated human immunodeficiency virus (HIV) is the etiological agent of the complex disease that includes progressive destruction of the immune system (acquired immune deficiency syndrome; AIDS) and degeneration of the central and peripheral nervous system. There are many strains of HIV. The two main ones are HIV-1 and HIV-2, the latter one producing a less severe disease than the first one. Being a retrovirus, its genetic material is in the form of RNA (ribonucleic acid) consisting of two RNA strands. Coexisting with RNA are reverse transcriptase, integrase, a protease, a ribonuclease and other enzymes.

It is known that some antiviral compounds which act as inhibitors of HIV replication are effective agents in the treatment of AIDS and similar diseases. , including reverse transcriptase inhibitors and protease inhibitors such as indinavir and nelfinavir. Drugs

that are known for the treatment of HIV-infected patients belong to the class of nucleoside reverse transcriptase (RT) inhibitors such as azidothyraidine (AZT), and lamivudine, non-nucleoside reverse transcriptase inhibitors such as nevirapine (Boehringer Ingelheim) efavirenz (DuPont) or protease inhibitors such as nelfinavir (Agouron), saquinavir (Roche), ritanovir (Abbott laboratories) and amprenavir (GlaxoWellcome). A new reverse transcriptase drug recently added in the batlle against HIV is tenofovir (PMPA, Gilead Sciences). A relatively new target that is focussed on lately is the integrase enzyme of HIV, while also many other proteins acting as enzymes or co-factors are being investigated.

Replication of the human immunodeficiency virus type 1 (hereinafter referred as HIV-1) can be drastically reduced in infected patients by combining potent antiviral drugs targeted at multiple viral targets, as reviewed by Vandamme et al. in *Antiviral Chem. Chemother.* (1998) 9:187-203.

Multiple-drug combination regimens can reduce viral load below the detection limit of the most sensitive tests. Nevertheless low level ongoing replication has been shown to occur, possibly in sanctuary sites, leading to the emergence of drug-resistant strains, according to Perelson et al. in *Nature* (1997) 387:123-124. Furthermore, the selectivity index of many anti-viral agents is rather low, thereby they are possibly responsible for side-effects and toxicity. Moreover, HIV can develop resistance to most, if not all, currently approved antiviral drugs, according to Schmit et al. in *J. Infect. Dis.* (1996) 174:962-968. It is well documented that the ability of HIV to rapidly evolve drug resistance, together with toxicity problems requires the development of additional classes of antiviral drugs.

As a summary, there is still a stringent need in the art for potent inhibitors of HIV. Therefore a goal of the present invention is to satisfy this urgent need by identifying efficient and non-harmful pharmaceutically active ingredients and combination of ingredients for the treatment of retroviral infections, in particular lentiviral infections, and more particularly HIV infections, in mammals and in humans. Furthermore, there is also a need for compounds which either complement existing drugs such that the resulting

cocktail has improved activity or resistance to virus mutation or compounds which are themselves effective against many or all viable mutations of a virus.

The present invention relates to new phosphonoalkoxy substituted nucleoside analogs comprising a phosphonalkoxy substituted five-membered, saturated or unsaturated, oxygen, nitrogen or sulfur containing ring coupled to a pyrimidine or purine base and to their use as anti-viral agents.

Pioneering work on the chemistry of phosphonate nucleosides has been carried out by A. Burger[1,2], while the work of D. Rammler[3,4], A. Holy[5] and J. Moffatt[6] has led to important new reaction schemes to synthesize phosphonate nucleosides. Phosphonate nucleosides can be devided in two categories. A first category of phosphonate nucleosides (1) are real nucleoside analogues as they contain a nucleobase and a sugar moiety.[3-6] A second series of phosphonate nucleosides represented by PMEA (2) can better be considered as alkylated nucleobases as the sugar moiety is replaced by an alkoxyalkyl moiety.[7] Surprisingly, up to now, potent antiviral *in* vivo activity (HSV, CMV, HBV, HIV) has only been associated with phosphonoalkoxyalkyl nucleobases and not with sugar containing phosphonate nucleosides. Several attempts to discover antiviral nucleoside phosphonates has led to synthetic schemes for the preparation of furanose[8], pyranose[9] and carbocyclic phosphonate nucleosides[10], lacking, however, potent antiviral activity.

Phosphorylation by kinases and incorporation in nucleic acids (eventually leading to chain termination) is considered as an important mechanism to explain the antiviral activity of nucleosides. The lack of antiviral activity of nucleoside phosphonates is generally explained by their poor substrate properties for cellular and viral kinases. On the other hand, the potent antiviral activity of phosphonylated alkylated nucleobases is ascribed to their intracellular phosphorylation into their diphosphates and to a refracting incorporation of the modified nucleosides in nucleic acids (the enzymatic incorporation in nucleic acids is, almost, irreversible).[11] A disadvantage of the acyclic nucleoside phosphonates are their low selectivity index in cellular screening systems.[7,11] The selectivity for HIV reverse transcriptase versus mitochondrial DNA polymerases of the triphosphates of anti-HIV nucleosides is an important factor determining *in* vivo toxicity.[12]

Threose nucleosides have been previously synthesized because they can be assembled from natural precursor molecules.[13] It has been demonstrated that threose nucleic acids (TNA) form duplexes with DNA and RNA of thermal stability, similar to that of the natural nucleic acids association.[13] Triphosphates of threose nucleosides are accepted as substrate by several polymerases and they can be enzymatically incorporated in DNA.[14,15] The phosphonoalkoxy group of the proposed furanose nucleoside phosphonates is bound at the 3'-position bringing the phosphorous atom and the nucleobase closer to each other than in previously synthesized nucleoside phosphonates.[8] The presence of an anomeric centre in threose nucleoside phosphonates gives them similar stereo-electronic properties than natural nucleosides.

SUMMARY OF THE INVENTION

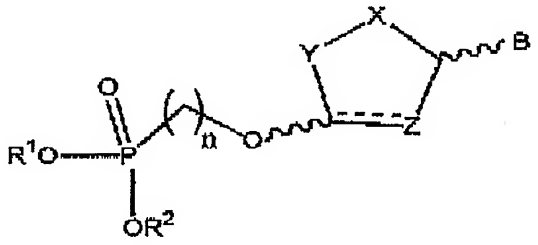
In the present invention, new anti-viral, more in particular anti-HIV compounds are provided. The compounds are novel phosphonate nucleosides, more in particular phosphonoalkoxy substituted nucleosides comprising a five-membered, saturated or unsaturated, oxygen, nitrogen or sulfur containing ring, or analogues or derivatives thereof and it has been shown that they possess anti-viral activity, more specifically against HIV. The present invention demonstrates that the compounds inhibit the replication of HIV. Therefore, these phosphonate nucleosides constitute a new potent class of anti-viral compounds that can be used in the treatment and prevention of viral infections in animals, mammals and humans, more specifically for the treatment and prevention of HIV.

The present invention relates to new phosphonate nucleosides, more in particular to phosphonoalkoxy substituted nucleosides comprising a five-membered, oxygen, nitrogen or sulfur containing, saturated or unsaturated ring, or analogues or derivatives thereof. The invention further relates to compounds having anti-viral activity, more specifically to novel phosphonate nucleosides having viral replication inhibiting properties, more in particular of HIV (Human Immunodeficiency Virus), which is the etiological agent of Acquired Immune Defiency Syndrome (AIDS) in humans, and consequently may be

useful for the treatment of individuals infected by HIV. The present invention also relates to compounds having antiviral activities with respect to other viruses, such as Hepatitis B Virus. Present invention furthermore relates to the use of the compounds as a medicine and more specifically to the use of the compounds as an anti-viral agent. The invention also relates to methods for preparation of all such compounds and pharmaceutical compositions comprising them. The invention further relates to the use of said compounds in the manufacture of a medicament useful for the treatment of subjects suffering from HIV infection, as well as for treatment of other viral, retroviral or lentiviral infections, treatment of animals suffering from FIV, viral, retroviral, lentiviral infections or treatment of tumour or cancer cells. The present invention also relates to a method of treatment or prevention of viral infections, by using said compounds.

One aspect of the present invention is the provision of new phosphonate nucleosides, said new phosphonate nucleosides comprising a phosphonoalkoxy substituted five-membered, saturated or unsaturated ring which is coupled to a pyrimidine or purine base. In a particular embodiment, the five-membered ring is an oxygen, nitrogen or sulfur containing ring and in a yet more particular embodiment, the five-membered ring is an oxygen containing ring.

The present invention relates to phosphonoalkoxy substituted nucleosides comprising a five-membered, saturated or unsaturated, oxygen, nitrogen or sulfur containing ring, or analogues or derivatives thereof corresponding to the formula I, isomers, solvates or pharmaceutical salts thereof,



(I)

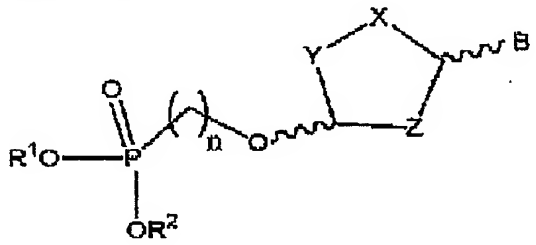
wherein:

- B is a heterocycle selected from the group consisting of pyrimidine and purine bases;

- The dotted line represents an optional double bond, provided that if Z is oxygen, or sulfur, there is no double bond;
- Each R^1 and R^2 are independently selected from the group of hydrogen; $(-PO_3R^6)_m$-$PO_3R^7R^8$; alkyl; alkenyl; alkynyl; cycloalkyl; cycloalkenyl; cycloalkynyl; aryl; arylalkyl; heterocyclic ring; heterocyclic ring-alkyl; acyloxyalkyl; acyloxyalkenyl; acyloxyalkynyl; acyloxyaryl; acyloxyarylalkyl; acyloxyarylalkenyl; acyloxyarylalkynyl; dialkylcarbonate; alkylarylcarbonate; alkylalkenylcarbonate; alkylalkynylcarbonate; alkenylarylcarbonate; alkynylarylcarbonate; alkenylalkynylcarbonate; dialkenylcarbonate; dialkynylcarbonate; wherein said alkyl, alkenyl and alkynyl can contain a heteroatom in or at the end of the hydrocarbon chain, said heteroatom selected from O, S and N and R^1 and R^2 can further be selected from substituents known for (anti-viral) phosphonates in order to create prodrugs;
- Each X, Y and Z are independently selected from the group of oxygen; nitrogen; sulfur; CHR^3; wherein at least one of X, Y or Z is oxygen, nitrogen or sulfur and maximally two of X, Y or Z are oxygen, nitrogen or sulfur and the other(s) is/are CHR^3;
- R^3 is selected from hydrogen; azido; F and OR^4;
- Each R^4, R6, R^7 and R^8 are independently selected from hydrogen; alkyl; alkenyl; alkynyl; cycloalkyl; cycloalkenyl; cycloalkynyl; aryl; arylalkyl; heterocyclic ring; heterocyclic ring-alkyl; acyloxyalkyl; wherein said alkyl, alkenyl and alkynyl can contain a heteroatom in or at the end of the hydrocarbon chain, said heteroatom selected from O, S and N;
- n is selected from 1 to 6;
- m is 0 or 1.

The groups R^1 and R^2 refers to all known phosphonate prodrugs and substituents known and used in the prior art for anti-viral phosphonate nucleosides such as described for example in US6225460, US5977089, WO0208241 and many other publications. Subsequently, the present invention also provides for the prodrugs of the phosphonate nucelosides of the present invention.

One embodiment of the present invention relates to phosphonoalkoxy substituted nucleosides comprising a five-membered, saturated or unsaturated, oxygen, nitrogen or sulfur containing ring, or analogues or derivatives thereof corresponding to the formula II, isomers, solvates or pharmaceutical salts thereof,



(II)

wherein:

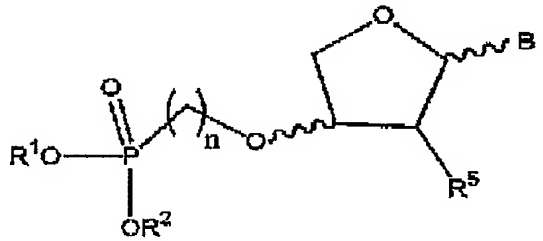
- B is a heterocycle selected from the group consisting of pyrimidine and purine bases;
- Each R^1 and R^2 are independently selected from the group of hydrogen; $(-PO_3R^6)_m$-$PO_3R^7R^8$; alkyl; alkenyl; alkynyl; cycloalkyl; cycloalkenyl; cycloalkynyl; aryl; arylalkyl; heterocyclic ring; heterocyclic ring-alkyl; acyloxyalkyl; acyloxyalkenyl; acyloxyalkynyl; acyloxyaryl; acyloxyarylalkyl; acyloxyarylalkenyl; acyloxyarylalkynyl; dialkylcarbonate; alkylarylcarbonate; alkylalkenylcarbonate; alkylalkynylcarbonate; alkenylarylcarbonate; alkynylarylcarbonate; alkenylalkynylcarbonate; dialkenylcarbonate; dialkynylcarbonate; wherein said alkyl, alkenyl and alkynyl can contain a heteroatom in or at the end of the hydrocarbon chain, said heteroatom selected from O, S and N and are further selected from substituents known for phosphonates described as anti-viral agents;
- Each X, Y and Z are independently selected from the group of oxygen; nitrogen; sulfur; CHR^3; wherein at least one of X, Y or Z is oxygen, nitrogen or sulfur and maximally two of X, Y or Z are oxygen, nitrogen or sulfur and the other(s) is/are CHR^3;
- R^3 is selected from hydrogen; azido; F and OR^4;
- R^4 is selected from hydrogen; alkyl; alkenyl; alkynyl; cycloalkyl; cycloalkenyl; cycloalkynyl; aryl; heterocyclic ring; arylalkyl; heterocyclic ring-alkyl; acyloxyalkyl; wherein said alkyl, alkenyl and alkynyl can contain a heteroatom in or at the end of the hydrocarbon chain, said heteroatom selected from O, S and N;

- Each R^6, R^7 and R^8 are independently selected from hydrogen; alkyl; alkenyl; alkynyl; cycloalkyl; cycloalkenyl; cycloalkynyl; aryl; arylalkyl; heterocyclic ring; heterocyclic ring-alkyl; acyloxyalkyl; wherein said alkyl, alkenyl and alkynyl can contain a heteroatom in or at the end of the hydrocarbon chain, said heteroatom selected from O, S and N;
- n is selected from 1, 2, 3, 4, 5 or 6;
- m is 0 or 1.

A particular embodiment provides compounds according to the formulas of the invention wherein the compounds comprise a five-membered, oxygen containing ring and each X, Y and Z are independently selected from the group of oxygen and CHR^3; wherein at least one of X, Y or Z is oxygen and maximally two of X, Y or Z are oxygen, and the other(s) is/are CHR^3. In another embodiment, the invention relates to compounds according to the formulas of the invention, wherein n is maximally 3.

Another particular embodiment relates to novel 3'-phosphonalkoxy substituted, saturated or unsaturated, furanose nucleosides comprising a purine or pyrimidine base coupled to the 1' position of a furanose whereby the 3' position of the furanose is substituted with a phosphonoalkoxy group. The nucleosides can also be derived of tetrahydrofuran or 3,4-dihydro-furan thereby substituted at the 2 postion with a heterocycle selected from the group consisting of pyrimidine and purine bases and at the 4 position with a phosphonoalkoxy group.

One embodiment of the present invention relates to 3'-phosphonate substituted furanose nucleosides according to formula (III), pharmaceutically acceptable salts, solvates, and isomers thereof,



(III)

wherein,

- B is a heterocycle selected from the group consisting of pyrimidine and purine bases;
- Each R^1 and R^2 are independently selected from the group of hydrogen; $(-PO_3R^6)_m$-$PO_3R^7R^8$; alkyl; alkenyl; alkynyl; cycloalkyl; cycloalkenyl; cycloalkynyl; aryl; arylalkyl; heterocyclic ring; heterocyclic ring-alkyl; acyloxyalkyl; acyloxyalkenyl; acyloxyalkynyl; acyloxyaryl; acyloxyarylalkyl; acyloxyarylalkenyl; acyloxyarylalkynyl; dialkylcarbonate; alkylarylcarbonate; alkylalkenylcarbonate; alkylalkynylcarbonate; alkenylarylcarbonate; alkynylarylcarbonate; alkenylalkynylcarbonate; dialkenylcarbonate; dialkynylcarbonate; wherein said alkyl, alkenyl and alkynyl can contain a heteroatom in or at the end of the hydrocarbon chain, said heteroatom selected from O, S and N and are further selected from substituents known for phosphonates described as anti-viral agents;
- R^5 is selected from hydrogen, azido, F and OR^4;
- R^4 is selected from hydrogen; alkyl; alkenyl; alkynyl; cycloalkyl; cycloalkenyl; cycloalkynyl; aryl; heterocyclic ring; arylalkyl; heterocyclic ring-alkyl; acyloxyalkyl; wherein said alkyl, alkenyl and alkynyl can contain a heteroatom in or at the end of the hydrocarbon chain, said heteroatom selected from O, S and N;
- Each R^6, R^7 and R^8 are independently selected from hydrogen; alkyl; alkenyl; alkynyl; cycloalkyl; cycloalkenyl; cycloalkynyl; aryl; arylalkyl; heterocyclic ring; heterocyclic ring-alkyl; acyloxyalkyl; wherein said alkyl, alkenyl and alkynyl can contain a heteroatom in or at the end of the hydrocarbon chain, said heteroatom selected from O, S and N;
- n is selected from 1, 2, 3, 4, 5 or 6;
- m is 0 or 1.

A particular embodiment of the present invention provides the phosphonate substituted nucleosides of the formula (I), (II) or (III) wherein B is selected from adenine and thymine. Another particular embodiment of the present invention provides novel 3'-phosphonate substituted threose nucleosides, more in particular 3'-phosphonalkoxy substituted threose nucleosides. In another particular embodiment of the present invention, the 3'-phosponalkoxy substituent or the purine or pyrimidine bases coupled to the ring of the compounds of the invention are in the R or S configuration.

The present invention relates in a particular embodiment to compounds selected from the group:

1-(N^6-benzoyladenin-9-yl)-2-O-benzoyl-3-O-(diisopropylphosphonomethyl)-L-threose **(11)**

1-(thymin-1-yl)-2-O-benzoyl-3-O-(diisopropylphosphonomethyl)-L-threose **(12)**

1-(uracil-1-yl)-2-O-benzoyl-3-O-(diisopropylphosphonomethyl)-L-threose **(13)**

1-(N^4-acetylcytosin-1-yl)-2-O-benzoyl-3-O-(diisopropylphosphonomethyl)-L-threose **(14)**

1-(adenin-9-yl)-3-O-(diisopropylphosphonomethyl)-L-threose **(15)**

1-(thymin-1-yl)-3-O-(diisopropylphosphonomethyl)-L-threose **(16)**

1-(uracil-1-yl)-3-O-(diisopropylphosphonomethyl)-L-threose **(17)**

1-(cytosin-1-yl)-3-O-(diisopropylphosphonomethyl)-L-threose **(18)**

1-(adenin-9-yl)-2-deoxy-3-O-(diisopropylphosphonomethyl)-L-threose **(19)**

1-(thymin-1-yl)-2-deoxy-3-O-(diisopropylphosphonomethyl)-L-threose **(20)**

1-(uracil-1-yl)-2-deoxy-3-O-(diisopropylphosphonomethyl)-L-threose **(21)**

1-(cytosin-1-yl)-2-deoxy-3-O-(diisopropylphosphonomethyl)-L-threose **(22)**

1-(adenin-9-yl)-3-O-(phosphonomethyl)-L-threose sodium salt **(3a)**

1-(thymin-1-yl)-3-O-(phosphonomethyl)-L-threose sodium salt **(3b)**

1-(uracil-1-yl)-3-O-(phosphonomethyl)-L-threose sodium salt **(3c)**

1-(cytosin-1-yl)-3-O-(phosphonomethyl)-L-threose sodium salt **(3d)**

1-(adenin-1-yl)-2-deoxy-3-O-(phosphonomethyl)-L-threose sodium salt **(3e)**

1-(thymin-1-yl)-2-deoxy-3-O-(phosphonomethyl)-L-threose sodium salt **(3f)**

1-(uracil-1-yl)-2-deoxy-3-O-(phosphonomethyl)-L-threose sodium salt **(3g)**

1-(cytidin-1-yl)-2-deoxy-3-O-(phosphonomethyl)-L-threose sodium salt **(3h)**

According to a second aspect, the invention relates to the use of phosphonate substituted nucleosides of the formula (I), (II) or (III) as antiviral compounds, more particularly as compounds active against HIV. The invention also relates to the use of phosphonate substituted nucleosides of the formula (I), (II) or (III) for the manufacture of a medicine or as a pharmaceutically active ingredient, especially as a virus replication inhibitor, preferably a retrovirus replication inhibitor, for instance for the manufacture of a

medicament or pharmaceutical composition having antiviral activity for the prevention and/or treatment of viral, preferably retroviral, infections in humans and mammals. The present invention further relates to a method of treatment of a viral infection, preferably a retroviral infection in a mammal, including a human, comprising administering to the mammal in need of such treatment a therapeutically effective amount of a compound of formula (I), (II) or (III) as an active ingredient, preferably in admixture with at least a pharmaceutically acceptable carrier.

The invention further relates to methods for the preparation of compounds of formula (I), (II) or (III). The process for preparing the phosphonoalkoxy substituted nucleosides of the present invention comprises the steps of selectively protecting the hydroxy functions present on the five-membered ring that can not react in the following step, reacting the remaining free hydroxy of the protected five-membered ring with protected phosphonylalkyl, followed by reaction with a pyrimidine or purine base, deprotection of the five-membered ring protecting groups and possible purine or pyrimidine base protecting groups, if necessary a deoxygenation step of the hydroxy funstions present on the five-membered ring and finally a deprotection of the phosphonate protecting groups.

The invention also relates to pharmaceutical compositions comprising the compounds of the invention according to formula (I), (II) or (III) in admixture with at least a pharmaceutically acceptable carrier, the active ingredient preferably being in a concentration range of about 0.1 to 100% by weight, and to the use of these derivatives namely as drugs useful for the treatment of subjects suffering from HIV infection.

The invention further relates to the use of a composition comprising (a) one or more derivatives of formula (I), (II), or (III), and (b) one or more viral inhibitors as biologically active agents in respective proportions such as to provide a synergistic effect against a viral infection, preferably a lentiviral infection and more preferably a retroviral infection in a mammal, for instance in the form of a combined preparation for simultaneous, separate or sequential use in retroviral infection therapy. Within the framework of this embodiment of the invention, the retroviral enzyme inhibitors used as a therapeutically

active ingredients (b) may belong to categories already known in the art and include, among others,

- HIV integrase inhibitors such as are known in the art
- Nucleoside and non-nucleoside reverse transcriptase inhibitors such as for instance delavirdine, dideoxyadenosine, foscamet sodium, stavudine, suramin sodium, zalcitabine, zidovudine, lamivudine, didanosine, nevirapine, PMPA, and the like,
- HIV protease inhibitors such as for instance sequinavir, ritonavir, indinavir, nelfinavir and the like.

The invention also relates to the compounds of the invention according to formula (I), (II) or (III) being used for inhibition of the proliferation of other viruses than HIV, preferably the inhibition of viral activity of hepatitis B virus, hepatitis C virus or flaviviruses, with in particular yellow fever virus or Dengue virus.

More generally, the invention relates to the compounds of formula (I), (II), or (III) being useful as agents having biological activity (preferably antiviral or antitumoral activity) or as diagnostic agents. Any of the uses mentioned with respect to the present invention may be restricted to a non-medical use, a non-therapeutic use, a non-diagnostic use, or exclusively an in vitro use, or a use related to cells remote from an animal.

Another aspect of the invention relates to a pharmaceutical composition comprising a phosphonalkoxy substituted nucleoside of the invention according to formula (I), (II) or (III), more in particular having antiviral activity, yet more in particular against HIV.
A further aspect of the invention provides for a method of treatment or prevention of a viral infection in a mammal, comprising administering to the mammal in need of such treatment a therapeutically effective amount of a phosphonalkoxy substituted nucleoside of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Structure of a phosphonate nucleoside (**1**), a phosphonoalkoxyalkyl nucleoside (**2**) and threosyl nucleoside phosphonates (**3**).

Figure 2: Structure of furan, dihydrofuran and tetrahydrofuran with their numbering.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

In each of the following definitions, the number of carbon atoms represents the maximum number of carbon atoms generally optimally present in the substituent or linker; it is understood that where otherwise indicated in the present application, the number of carbon atoms represents the optimal maximum number of carbon atoms for that particular substituent or linker.

Examples of five-membered, oxygen, nitrogen or sulfur containing rings include but are not limited to tetrahydrofuran, dihydrofuran, dioxolane ([1,2]dioxolane, [1,3]dioxolane), oxathiolanes, isoxazolidines, and 3*H*-[1,2]dioxole.

The term "furanose" refers to five-membered cyclic monosaccharides. The numbering of monosaccharides starts at the carbon next to the oxygen inclosed in the ring and is indicated with a prime ('). The term "furan", "dihydrofuran" or "tetrahydrofuran" refers to a five-membered ring containing one oxygen atom with different saturations. They are exemplified in figure 2, together with their numbering.

The term "phosphonalkoxy" refers to a phosphonate coupled via an alkyl to an oxygen which can be coupled to another molecule.

The phosphonalkoxy substituted nucleosides of the invention are five-membered, saturated or unsaturated, oxygen, nitrogen or sulfur containing rings whereby a purine or pyrimidine base is coupled to a position of the ring and the 3-position of the five-membered ring, relative to the coupling position of the base, is substituted with a phosphonoalkoxy group.

The term "3'-phosphonalkoxy furanose nucleoside" refers to a purine or pyrimidine base coupled to the 1' position of a furanose whereby the 3' position of the furanose is substituted with a phosphonoalkoxy group.

The term "pyrimidine and purine bases" or "heterocycle selected from the group consisting of pyrimidine and purine bases" include but are not limited to adenine, thymine, cytosine, uracyl, guanine and (2,6-)diaminopurine and analogues thereof. A purine or pyrimidine base is a purine or pyrimidine base found in naturally occurring nucleosides as mentioned above. An analogue thereof is a base which mimics such naturally occurring bases in that their structures (the kinds of atoms and their arrangement) are similar to the naturally occurring bases but may either possess additional or lack certain of the functional properties of the naturally occurring bases. Such analogues include those derived by replacement of a CH moiety by a nitrogen atom, e.g. 5-azapyrimidines such as 5-azacytosine) or vice versa (e.g., 7-deazapurines, such as 7-deazaadenine or 7-deazaguanine) or both (e.g., 7-deaza, 8-azapurines). By derivatives of such bases or analogues are meant those bases wherein ring substituents are either incorporated, removed, or modified by conventional substituents known in the art, e.g., halogen, hydroxyl, amino, C_{1-6} alkyl. Such purine or pyrimidine bases, analogues and derivatives are well known to those skilled in the art. In a particular embodiment, the term "pyrimidine and purine bases" or "heterocycle selected from the group consisting of pyrimidine and purine bases" refers to adenine, thymine, cytosine, uracyl and guanine.

The term "alkyl" as used herein refers to C1-C18 normal, secondary, or tertiary hydrocarbon chains. Examples are methyl, ethyl, 1-propyl, 2-propyl, 1-butyl, 2-methyl-1-propyl(i-Bu), 2-butyl (s-Bu) 2-methyl-2-propyl (t-Bu), 1-pentyl (*n*-pentyl), 2-pentyl, 3-pentyl, 2-methyl-2-butyl, 3-methyl-2-butyl, 3-methyl-1-butyl, 2-methyl-1-butyl, 1-hexyl, 2-hexyl, 3-hexyl, 2-methyl-2-pentyl, 3-methyl-2-pentyl, 4-methyl-2-pentyl, 3-methyl-3-pentyl, 2-methyl-3-pentyl, 2,3-dimethyl-2-butyl, 3,3-dimethyl-2-butyl, *n*-pentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, *n*-decyl, *n*-undecyl, *n*-dodecyl, *n*-tridecyl, *n*-tetradecyl, *n*-pentadecyl, *n*-hexadecyl, *n*-heptadecyl, *n*-octadecyl, *n*-nonadecyl and *n*-icosyl.

As used herein and unless otherwise stated, the term "cycloalkyl" means a monocyclic saturated hydrocarbon monovalent radical having from 3 to 10 carbon atoms, such as for instance cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl and

the like, or a C_{7-10} polycyclic saturated hydrocarbon monovalent radical having from 7 to 10 carbon atoms such as, for instance, norbornyl, fenchyl, trimethyltricycloheptyl or adamantyl.

As used herein and unless otherwise stated, the term "cycloalkylene" refers to a cyclic hydrocarbon radical of 3-10 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkane; i.e. the divalent hydrocarbon radical corresponding to the above defined C_{3-10} cycloalkyl.

The terms "alkenyl" and "cycloalkenyl" as used herein is C2-C18 normal, secondary or tertiary and respectively C3-10 cyclic hydrocarbon with at least one site (usually 1 to 3, preferably 1) of unsaturation, i.e. a carbon-carbon, sp2 double bond. Examples include, but are not limited to: ethylene or vinyl (-CH=CH2), allyl (-CH2CH=CH2), cyclopentenyl (-C5H7), and 5-hexenyl (-CH2 CH2CH2CH2CH=CH2). The double bond may be in the cis or trans configuration.

The terms "alkynyl" and "cycloalkynyl" as used herein refer respectively C2-C18 normal, secondary, tertiary or the C3-10 cyclic hydrocarbon with at least one site (usually 1 to 3, preferably 1) of unsaturation, i.e. a carbon-carbon, sp triple bond. Examples include, but are not limited to: acetylenic (-C°CH) and propargyl (-CH2C°CH).

The terms " C_{1-18} alkylene" as used herein each refer to a saturated, branched or straight chain hydrocarbon radical of 1-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkane. Typical alkylene radicals include, but are not limited to: methylene (-CH2-) 1,2-ethyl (-CH2CH2-), 1,3-propyl (-CH2CH2CH2-), 1,4-butyl (-CH2CH2CH2CH2-), and the like.

The terms "alkenylene" and "cycloalkenylene" as used herein refer to an unsaturated branched chain, straight chain, and respectively a cyclic hydrocarbon radical of 2-18 respectively 3-10 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkene, i.e. double carbon-carbon bond moiety. Typical alkenylene radicals include, but are not limited to: 1,2-ethylene (-CH=CH-).

The terms " alkynylene" and "cycloalkynylene" as used herein refer respectively to an unsaturated, branched or straight chain of 2-18 carbon atoms or to a cyclic hydrocarbon radical of 3-10 carbon atoms respectively, having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkyne, i.e. triple carbon-carbon bond moiety. Typical alkynylene radicals include, but are not limited to: acetylene (-C°C-), propargyl (-CH2C°C-), and 4-pentynyl (-CH2CH2CH2C°CH-).

The term "aryl" as used herein means a aromatic hydrocarbon radical of 6-20 carbon atoms derived by the removal of hydrogen from a carbon atom of a parent aromatic ring system. Typical aryl groups include, but are not limited to 1 ring, or 2 or 3 rings fused together, radicals derived from benzene, naphthalene, spiro, anthracene, biphenyl, and the like.

"Arylalkyl" as used herein refers to an alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp3 carbon atom, is replaced with an aryl radical. Typical arylalkyl groups include, but are not limited to, benzyl, 2-phenylethan-1-yl, 2-phenylethen-1-yl, naphthylmethyl, 2-naphthylethan-1-yl, 2-naphthylethen-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like. The arylalkyl group comprises 6 to 20 carbon atoms, e.g. the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the arylalkyl group is 1 to 6 carbon atoms and the aryl moiety is 5 to 14 carbon atoms.

The term "heterocyclic ring" as used herein means pyridyl, dihydroypyridyl, tetrahydropyridyl (piperidyl), thiazolyl, tetrahydrothiophenyl, sulfur oxidized tetrahydrothiophenyl, furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, benzofuranyl, thianaphthalenyl, indolyl, indolenyl, quinolinyl, isoquinolinyl, benzimidazolyl, piperidinyl, 4-piperidonyl, pyrrolidinyl, 2-pyrrolidonyl, pyrrolinyl, tetrahydrofuranyl, bis-tetrahydrofuranyl, tetrahydropyranyl, bis-tetrahydropyranyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, octahydroisoquinolinyl, azocinyl, triazinyl, 6H-1,2,5-thiadiazinyl, 2H,6H-1,5,2-dithiazinyl, thianthrenyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, phenoxathinyl, 2H-pyrrolyl, isothiazolyl, isoxazolyl, pyrazinyl, pyridazinyl, indolizinyl, isoindolyl, 3H-indolyl, 1H-indazoly, purinyl, 4H-quinolizinyl, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, 4aH-carbazolyl, carbazolyl, ß-

carbolinyl, phenanthridinyl, acridinyl, pyrimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, furazanyl, phenoxazinyl, isochromanyl, chromanyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, pyrazolinyl, piperazinyl, indolinyl, isoindolinyl, quinuclidinyl, morpholinyl, oxazolidinyl, benzotriazolyl, benzisoxazolyl, oxindolyl, benzoxazolinyl, benzothienyl, benzothiazolyl and isatinoyl.

Heteroaryl means pyridyl, dihydropyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, s-triazinyl, oxazolyl, imidazolyl, thiazolyl, isoxazolyl, pyrazolyl, isothiazolyl, furanyl, thiofuranyl, thienyl, and pyrrolyl.

By way of example, carbon bonded heterocyclic rings are bonded at position 2, 3, 4, 5, or 6 of a pyridine, position 3, 4, 5, or 6 of a pyridazine, position 2, 4, 5, or 6 of a pyrimidine, position 2, 3, 5, or 6 of a pyrazine, position 2, 3, 4, or 5 of a furan, tetrahydrofuran, thiofuran, thiophene, pyrrole or tetrahydropyrrole, position 2, 4, or 5 of an oxazole, imidazole or thiazole, position 3, 4, or 5 of an isoxazole, pyrazole, or isothiazole, position 2 or 3 of an aziridine, position 2, 3, or 4 of an azetidine, position 2, 3, 4, 5, 6, 7, or 8 of a quinoline or position 1, 3, 4, 5, 6, 7, or 8 of an isoquinoline. Still more typically, carbon bonded heterocycles include 2-pyridyl, 3-pyridyl, 4-pyridyl, 5-pyridyl, 6-pyridyl, 3-pyridazinyl, 4-pyridazinyl, 5-pyridazinyl, 6-pyridazinyl, 2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 6-pyrimidinyl, 2-pyrazinyl, 3-pyrazinyl, 5-pyrazinyl, 6-pyrazinyl, 2-thiazolyl, 4-thiazolyl, or 5-thiazolyl.

By way of example, nitrogen bonded heterocyclic rings are bonded at position 1 of an aziridine, azetidine, pyrrole, pyrrolidine, 2-pyrroline, 3-pyrroline, imidazole, imidazolidine, 2-imidazoline, 3-imidazoline, pyrazole, pyrazoline, 2-pyrazoline, 3-pyrazoline, piperidine, piperazine, indole, indoline, 1H-indazole, position 2 of a isoindole, or isoindoline, position 4 of a morpholine, and position 9 of a carbazole, or β-carboline. Still more typically, nitrogen bonded heterocycles include 1-aziridyl, 1-azetedyl, 1-pyrrolyl, 1-imidazolyl, 1-pyrazolyl, and 1-piperidinyl.

"Carbocycle" means a saturated, unsaturated or aromatic ring system having 3 to 7 carbon atoms as a monocycle or 7 to 12 carbon atoms as a bicycle. Monocyclic carbocycles have 3 to 6 ring atoms, still more typically 5 or 6 ring atoms. Bicyclic carbocycles have 7 to 12 ring atoms, e.g. arranged as a bicyclo [4,5], [5,5], [5,6] or [6,6] system, or 9 or 10 ring atoms arranged as a bicyclo [5,6] or [6,6] system. Examples of

monocyclic carbocycles include cyclopropyl, cyclobutyl, cyclopentyl, 1-cyclopent-1-enyl, 1-cyclopent-2-enyl, 1-cyclopent-3-enyl, cyclohexyl, 1-cyclohex-1-enyl, 1-cyclohex-2-enyl, 1-cyclohex-3-enyl, phenyl, spiryl and naphthyl. Carbocycle thus includes some aryl groups.

The term "acyl" as used herein refers to substituted C(O), such as C(O)(alkyl, alkenyl, alkynyl, aryl, heterocyclic ring, arylalkyl, arylalkenyl, arylalkynyl, heterocyclic ring-alkyl, heterocyclic ring-alkenyl or heterocyclic ring-alkynyl) such as for example an alkanoyl group (alkylcarbonyl, alkyl coupled to a carbonyl), an aroyl group (arylcarbonyl, aryl attached to a carbonyl), a arylalkanoyl or a alkylaroyl group, wherein the C(O) is coupled to another molecule or atom and wherein said alkyl, alkenyl and alkynyl can contain a heteroatom in or at the end of the hydrocarbon chain, said heteroatom selected from O, S and N. As an example the term "acyloxyalkyl" refers to an acyl, coupled via an oxygen to alkyl, wherein the alkyl will be further coupled to another atom.

As an example, "alkylalkenylcarbonate" refers to a alkyl-OC(O)O-alkenyl group, thus a carbonate substituted at one side with an alkyl and on the other side with an alkenyl. One of the alkyl or alkenyl will be further coupled to another atom.

As used herein and unless otherwise stated, the terms " C_{1-18} alkoxy ", " C_{3-10} cycloalkoxy ", " aryloxy", "arylalkyloxy ", " oxyheterocyclic ring", "thio C_{1-7} alkyl", " thio C_{3-10} cycloalkyl ", " arylthio ", " arylalkylthio " and " thioheterocyclic ring" refer to substituents wherein a C_{1-18} alkyl radical, respectively a C_{3-10} cycloalkyl, aryl, arylalkyl or heterocyclic ring radical (each of them such as defined herein), are attached to an oxygen atom or a sulfur atom through a single bond, such as but not limited to methoxy, ethoxy, propoxy, butoxy, thioethyl, thiomethyl, phenyloxy, benzyloxy, mercaptobenzyl and the like.

As used herein and unless otherwise stated, the term halogen means any atom selected from the group consisting of fluorine (F), chlorine (Cl), bromine (Br) and iodine (I).

Any substituent designation that is found in more than one site in a compound of this invention shall be independently selected.

Substituents optionally are designated with or without bonds. Regardless of bond indications, if a substituent is polyvalent (based on its position in the structure referred to), then any and all possible orientations of the substituent are intended.

The compounds of the invention optionally are bound covalently to an insoluble matrix and used for affinity chromatography (separations, depending on the nature of the groups of the compounds, for example compounds with aryl are useful in hydrophobic affinity separations.

The compounds of the invention are employed for the treatment or prophylaxis of viral infections, more particularly HIV infections. When using one or more compounds according to the formulas of the application like (I), (II), (III) as defined herein:

- the active ingredients of the compound(s) may be administered to the mammal (including a human) to be treated by any means well known in the art, i.e. orally, intranasally, subcutaneously, intramuscularly, intradermally, intravenously, intra-arterially, parenterally or by catheterization.
- the therapeutically effective amount of the preparation of the compound(s), especially for the treatment of viral infections in humans and other mammals, preferably is a retroviral enzyme inhibiting amount. More preferably, it is a retroviral replication inhibiting amount or a retroviral enzyme inhibiting amount of the derivative(s) of formula (I), (II) or (III) as defined herein corresponds to an amount which ensures a plasma level of between 1μg/ml and 100 mg/ml, optionally of 10 mg/ml. This can be achieved by administration of a dosage of in the range of 0.001 mg to 20 mg, preferably 0.01 mg to 5 mg, preferably 0.1mg to 1 mg per day per kg bodyweight for humans. Depending upon the pathologic condition to be treated and the patient's condition, the said effective amount may be divided into several sub-units per day or may be administered at more than one day intervals.

The present invention further relates to a method for preventing or treating a viral infections in a subject or patient by administering to the patient in need thereof a therapeutically effective amount of phosphonate nucleosides of the present invention.

The therapeutically effective amount of the preparation of the compound(s), especially for the treatment of viral infections in humans and other mammals, preferably is HIV protein/enzyme inhibiting amount. More preferably, it is a HIV replication inhibiting amount or a HIV enzyme inhibiting amount of the derivative(s) of the formulas as defined herein. Suitable dosage is usually in the range of 0.001 mg to 20 mg, preferably 0.01 mg to 5 mg, preferably 0.1mg to 1 mg per day per kg bodyweight for humans. Depending upon the pathologic condition to be treated and the patient's condition, the said effective amount may be divided into several sub-units per day or may be administered at more than one day intervals.

As is conventional in the art, the evaluation of a synergistic effect in a drug combination may be made by analyzing the quantification of the interactions between individual drugs, using the median effect principle described by Chou et al. in *Adv. Enzyme Reg.* (1984) 22:27. Briefly, this principle states that interactions (synergism, additivity, antagonism) between two drugs can be quantified using the combination index (hereinafter referred as CI) defined by the following equation:

$$CI_x = \frac{ED_x^{1c}}{ED_x^{1a}} + \frac{ED_x^{2c}}{ED_x^{2a}}$$

wherein ED_x is the dose of the first or respectively second drug used alone (1a, 2a), or in combination with the second or respectively first drug (1c, 2c), which is needed to produce a given effect. The said first and second drug have synergistic or additive or antagonistic effects depending upon CI < 1, CI = 1, or CI > 1, respectively.

Synergistic activity of the pharmaceutical compositions or combined preparations of this invention against viral infection may also be readily determined by means of one or more tests such as, but not limited to, the isobologram method, as previously described by Elion et al. in *J. Biol. Chem.* (1954) 208:477-488 and by Baba et al. in *Antimicrob. Agents Chemother.* (1984) 25:515-517, using EC_{50} for calculating the fractional inhibitory concentration (hereinafter referred as FIC). When the minimum FIC index corresponding to the FIC of combined compounds (e.g., $FIC_x + FIC_y$) is equal to 1.0, the combination is said to be additive; when it is beween 1.0 and 0.5, the combination is

defined as subsynergistic, and when it is lower than 0.5, the combination is defined as synergistic. When the minimum FIC index is between 1.0 and 2.0, the combination is defined as subantagonistic and, when it is higher than 2.0, the combination is defined as antagonistic.

This principle may be applied to a combination of different antiviral drugs of the invention or to a combination of the antiviral drugs of the invention with other drugs that exhibit anti-HIV activity.

The invention thus relates to a pharmaceutical composition or combined preparation having synergistic effects against a viral infection and containing:

Either:

A)

(a) a combination of two or more of the phosphonate nucleosides of the present invention, and

(b) optionally one or more pharmaceutical excipients or pharmaceutically acceptable carriers,

for simultaneous, separate or sequential use in the treatment or prevention of a viral infection

or

B)

(c) one or more anti-viral agents, and

(d) at least one of the phosphonate nucleosides of the present invention, and

(e) optionally one or more pharmaceutical excipients or pharmaceutically acceptable carriers,

for simultaneous, separate or sequential use in the treatment or prevention of a viral infection.

Suitable anti-viral agents for inclusion into the synergistic antiviral compositions or combined preparations of this invention include practically all known anti-HIV compounds known at this moment such as nucleoside and non-nucleoside reverse transcriptase inhibitors, protease inhibitors and integrase inhibitors.

The pharmaceutical composition or combined preparation with synergistic activity against viral infection according to this invention may contain the phosphonate nucleosides of the present invention, compounds according to the formulas of the application like (I), (II), (III), over a broad content range depending on the contemplated use and the expected effect of the preparation. Generally, the content of the phosphonate nucleosides of the present invention of the combined preparation is within the range of 0.1 to 99.9% by weight, preferably from 1 to 99% by weight, more preferably from 5 to 95% by weight.

According to a particular embodiment of the invention, the compounds of the invention may be employed in combination with other therapeutic agents for the treatment or prophylaxis of HIV infections. The invention therefore relates to the use of a composition comprising:

(a) one or more compounds of formula (I), (II), or (III), and

(b) one or more HIV /protein-enzyme inhibitors as biologically active agents in respective proportions such as to provide a synergistic effect against a viral infection, particularly a HIV infection in a mammal, for instance in the form of a combined preparation for simultaneous, separate or sequential use in viral infection therapy, such as HIV.

When using a combined preparation of (a) and (b):

- the active ingredients (a) and (b) may be administered to the mammal (including a human) to be treated by any means well known in the art, i.e. orally, intranasally, subcutaneously, intramuscularly, intradermally, intravenously, intra-arterially, parenterally or by catheterization.
- the therapeutically effective amount of the combined preparation of (a) and (b), especially for the treatment of viral infections in humans and other mammals, particularly is a HIV enzyme inhibiting amount. More particularly, it is a HIV replication inhibiting amount of derivative (a) and a HIV enzyme inhibiting amount of inhibitor (b). Still more particularly when the said HIV enzyme inhibitor (b) is a reverse transcriptase inhibitor, its effective amount is a reverse transcriptase

inhibiting amount. When the said HIV enzyme inhibitor (b) is a protease inhibitor, its effective amount is a protease inhibiting amount.

- ingredients (a) and (b) may be administered simultaneously but it is also beneficial to administer them separately or sequentially, for instance within a relatively short period of time (e.g. within about 24 hours) in order to achieve their functional fusion in the body to be treated.

The invention also relates to the compounds of the invention, compounds according to the formulas of the application like (I), (II), (III) being used for inhibition of the proliferation of other viruses than HIV, particularly for the inhibition of other retroviruses and lentiviruses and also for the inhibition of flaviviruses or picornaviruses such as BVDV, HCV, HBV or Coxsackie virus, with in particular yellow fever virus, Dengue virus, hepatitis B virus, hepatitis G virus, Classical Swine Fever virus or the Border Disease Virus. Als other viruses may be inhibited such as HSV, CMV and Sars-virus.

The present invention further provides veterinary compositions comprising at least one active ingredient as above defined together with a veterinary carrier therefore. Veterinary carriers are materials useful for the purpose of administering the composition and may be solid, liquid or gaseous materials which are otherwise inert or acceptable in the veterinary art and are compatible with the active ingredient. These veterinary compositions may be administered orally, parenterally or by any other desired route.

More generally, the invention relates to the compounds according to the formulas of the application like (I), (II), (III) being useful as agents having biological activity (particularly antiviral activity) or as diagnostic agents. Any of the uses mentioned with respect to the present invention may be restricted to a non-medical use, a non-therapeutic use, a non-diagnostic use, or exclusively an in vitro use, or a use related to cells remote from an animal.

Those of skill in the art will also recognize that the compounds of the invention may exist in many different protonation states, depending on, among other things, the pH of their environment. While the structural formulae provided herein depict the compounds in only

one of several possible protonation states, it will be understood that these structures are illustrative only, and that the invention is not limited to any particular protonation state, any and all protonated forms of the compounds are intended to fall within the scope of the invention.

The term "pharmaceutically acceptable salts" as used herein means the therapeutically active non-toxic salt forms which the compounds according to the formulas of the application like (I), (II), (III) are able to form. Therefore, the compounds of this invention optionally comprise salts of the compounds herein, especially pharmaceutically acceptable non-toxic salts containing, for example, Na+, Li+, K+, Ca+2 and Mg+2. Such salts may include those derived by combination of appropriate cations such as alkali and alkaline earth metal ions or ammonium and quaternary amino ions with an acid anion moiety, typically a carboxylic acid. The compounds of the invention may bear multiple positive or negative charges. The net charge of the compounds of the invention may be either positive or negative. Any associated counter ions are typically dictated by the synthesis and/or isolation methods by which the compounds are obtained. Typical counter ions include, but are not limited to ammonium, sodium, potassium, lithium, halides, acetate, trifluoroacetate, etc., and mixtures thereof. It will be understood that the identity of any associated counter ion is not a critical feature of the invention, and that the invention encompasses the compounds in association with any type of counter ion. Moreover, as the compounds can exist in a variety of different forms, the invention is intended to encompass not only forms of the compounds that are in association with counter ions (e.g., dry salts), but also forms that are not in association with counter ions (e.g., aqueous or organic solutions). Metal salts typically are prepared by reacting the metal hydroxide with a compound of this invention. Examples of metal salts which are prepared in this way are salts containing Li+, Na+, and K+. A less soluble metal salt can be precipitated from the solution of a more soluble salt by addition of the suitable metal compound. In addition, salts may be formed from acid addition of certain organic and inorganic acids to basic centers, typically amines, or to acidic groups. Examples of such appropriate acids include, for instance, inorganic acids such as hydrohalic acids, e.g. hydrochloric or hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like;

or organic acids such as, for example, acetic, propanoic, hydroxyacetic, 2-hydroxypropanoic, 2-oxopropanoic, lactic, pyruvic, oxalic (i.e. ethanedioic), malonic, succinic (i.e. butanedioic acid), maleic, fumaric, malic, tartaric, citric, methanesulfonic, ethanesulfonic, benzenesulfonic, p-toluenesulfonic, cyclohexanesulfamic, salicylic (i.e. 2-hydroxybenzoic), p-aminosalicylic and the like. Furthermore, this term also includes the solvates which the compounds according to the formulas of the application like (I), (II), (III) as well as their salts are able to form, such as for example hydrates, alcoholates and the like. Finally, it is to be understood that the compositions herein comprise compounds of the invention in their unionized, as well as zwitterionic form, and combinations with stoichiometric amounts of water as in hydrates.

Also included within the scope of this invention are the salts of the parental compounds with one or more amino acids, especially the naturally-occurring amino acids found as protein components. The amino acid typically is one bearing a side chain with a basic or acidic group, e.g., lysine, arginine or glutamic acid, or a neutral group such as glycine, serine, threonine, alanine, isoleucine, or leucine.

The compounds of the invention also include physiologically acceptable salts thereof. Examples of physiologically acceptable salts of the compounds of the invention include salts derived from an appropriate base, such as an alkali metal (for example, sodium), an alkaline earth (for example, magnesium), ammonium and NX4+ (wherein X is C1-C4 alkyl). Physiologically acceptable salts of an hydrogen atom or an amino group include salts of organic carboxylic acids such as acetic, benzoic, lactic, fumaric, tartaric, maleic, malonic, malic, isethionic, lactobionic and succinic acids; organic sulfonic acids, such as methanesulfonic, ethanesulfonic, benzenesulfonic and p-toluenesulfonic acids; and inorganic acids, such as hydrochloric, sulfuric, phosphoric and sulfamic acids. Physiologically acceptable salts of a compound containing a hydroxy group include the anion of said compound in combination with a suitable cation such as Na+ and NX4+ (wherein X typically is independently selected from H or a C1-C4 alkyl group). However, salts of acids or bases which are not physiologically acceptable may also find use, for example, in the preparation or purification of a physiologically acceptable compound. All salts, whether or not derived form a physiologically acceptable acid or base, are within the scope of the present invention.

As used herein and unless otherwise stated, the term "enantiomer" means each individual optically active form of a compound of the invention, having an optical purity or enantiomeric excess (as determined by methods standard in the art) of at least 80% (i.e. at least 90% of one enantiomer and at most 10% of the other enantiomer), preferably at least 90% and more preferably at least 98%.

The term "isomers" as used herein means all possible isomeric forms, including tautomeric and sterochemical forms, which the compounds according to the formulas of the application like (I), (II), (III) may possess, but not including position isomers. Typically, the structures shown herein exemplify only one tautomeric or resonance form of the compounds, but the corresponding alternative configurations are contemplated as well. Unless otherwise stated, the chemical designation of compounds denotes the mixture of all possible stereochemically isomeric forms, said mixtures containing all diastereomers and enantiorners (since the compounds according to the formulas of the application like (I), (II), (III) may have at least one chiral center) of the basic molecular structure, as wel as the stereochemically pure or enriched compounds. More particularly, stereogenic centers may have either the R- or S-configuration, and multiple bonds may have either cis- or trans-configuration.

Pure isomeric forms of the said compounds are defined as isomers substantially free of other enantiomeric or diastereomeric forms of the same basic molecular structure. In particular, the term "stereoisomerically pure" or "chirally pure" relates to compounds having a stereoisomeric excess of at least about 80% (i.e. at least 90% of one isomer and at most 10% of the other possible isomers), preferably at least 90%, more preferably at least 94% and most preferably at least 97%. The terms "enantiomerically pure" and "diastereomerically pure" should be understood in a similar way, having regard to the enantiomeric excess, respectively the diastereomeric excess, of the mixture in question.

Separation of stereoisomers is accomplished by standard methods known to those in the art. One enantiomer of a compound of the invention can be separated substantially free of its opposing enantiomer by a method such as formation of diastereomers using optically active resolving agents ("Stereochemistry of Carbon Compounds," (1962) by E. L. Eliel, McGraw Hill; Lochmuller, C. H., (1975) J. Chromatogr., 113:(3) 283-302). Separation of

isomers in a mixture can be accomplished by any suitable method, including: (1) formation of ionic, diastereomeric salts with chiral compounds and separation by fractional crystallization or other methods, (2) formation of diastereomeric compounds with chiral derivatizing reagents, separation of the diastereomers, and conversion to the pure enantiomers, or (3) enantiomers can be separated directly under chiral conditions. Under method (1), diastereomeric salts can be formed by reaction of enantiomerically pure chiral bases such as brucine, quinine, ephedrine, strychnine, a-methyl-b-phenylethylamine (amphetamine), and the like with asymmetric compounds bearing acidic functionality, such as carboxylic acid and sulfonic acid. The diastereomeric salts may be induced to separate by fractional crystallization or ionic chromatography. For separation of the optical isomers of amino compounds, addition of chiral carboxylic or sulfonic acids, such as camphorsulfonic acid, tartaric acid, mandelic acid, or lactic acid can result in formation of the diastereomeric salts. Alternatively, by method (2), the substrate to be resolved may be reacted with one enantiomer of a chiral compound to form a diastereomeric pair (Eliel, E. and Wilen, S. (1994) Stereochemistry of Organic Compounds, John Wiley & Sons, Inc., p. 322). Diastereomeric compounds can be formed by reacting asymmetric compounds with enantiomerically pure chiral derivatizing reagents, such as menthyl derivatives, followed by separation of the diastereomers and hydrolysis to yield the free, enantiomerically enriched compounds of the invention. A method of determining optical purity involves making chiral esters, such as a menthyl ester or Mosher ester, a-methoxy-a-(trifluoromethyl)phenyl acetate (Jacob III. (1982) J. Org. Chem. 47:4165), of the racemic mixture, and analyzing the NMR spectrum for the presence of the two atropisomeric diastereomers. Stable diastereomers can be separated and isolated by normal- and reverse-phase chromatography following methods for separation of atropisomeric naphthyl-isoquinolines (Hoye, T., WO 96/15111).Under method (3), a racemic mixture of two asymmetric enantiomers is separated by chromatography using a chiral stationary phase. Suitable chiral stationary phases are, for example, polysaccharides, in particular cellulose or amylose derivatives. Commercially available polysaccharide based chiral stationary phases are ChiralCel™ CA, OA, OB5, OC5, OD, OF, OG, OJ and OK, and ChiralpakTM AD, AS, OP(+) and OT(+). Appropriate eluents or mobile phases for use in combination with said polysaccharide

chiral stationary phases are hexane and the like, modified with an alcohol such as ethanol, isopropanol and the like. ("Chiral Liquid Chromatography" (1989) W. J. Lough, Ed. Chapman and Hall, New York; Okamoto, (1990) "Optical resolution of dihydropyridine enantiomers by High-performance liquid chromatography using phenylcarbamates of polysaccharides as a chiral stationary phase", J. of Chromatogr. 513:375-378).
The terms cis and trans are used herein in accordance with Chemical Abstracts nomenclature and include reference to the position of the substituents on a ring moiety. The absolute stereochemical configuration of the compounds according to the formulas of the application like (I), (II), (III) may easily be determined by those skilled in the art while using well-known methods such as, for example, X-ray diffraction or NMR.

The compounds of the invention may be formulated with conventional carriers and excipients, which will be selected in accord with ordinary practice. Tablets will contain excipients, glidants, fillers, binders and the like. Aqueous formulations are prepared in sterile form, and when intended for delivery by other than oral administration generally will be isotonic. Formulations optionally contain excipients such as those set forth in the "Handbook of Pharmaceutical Excipients" (1986) and include ascorbic acid and other antioxidants, chelating agents such as EDTA, carbohydrates such as dextrin, hydroxyalkylcellulose, hydroxyalkylmethylcellulose, stearic acid and the like.
Subsequently, the term "pharmaceutically acceptable carrier" as used herein means any material or substance with which the active ingredient is formulated in order to facilitate its application or dissemination to the locus to be treated, for instance by dissolving, dispersing or diffusing the said composition, and/or to facilitate its storage, transport or handling without impairing its effectiveness. The pharmaceutically acceptable carrier may be a solid or a liquid or a gas which has been compressed to form a liquid, i.e. the compositions of this invention can suitably be used as concentrates, emulsions, solutions, granulates, dusts, sprays, aerosols, suspensions, ointments, creams, tablets, pellets or powders.
Suitable pharmaceutical carriers for use in the said pharmaceutical compositions and their formulation are well known to those skilled in the art, and there is no particular restriction to their selection within the present invention. They may also include additives such as

wetting agents, dispersing agents, stickers, adhesives, emulsifying agents, solvents, coatings, antibacterial and antifungal agents (for example phenol, sorbic acid, chlorobutanol), isotonic agents (such as sugars or sodium chloride) and the like, provided the same are consistent with pharmaceutical practice, i.e. carriers and additives which do not create permanent damage to mammals. The pharmaceutical compositions of the present invention may be prepared in any known manner, for instance by homogeneously mixing, coating and/or grinding the active ingredients, in a one-step or multi-steps procedure, with the selected carrier material and, where appropriate, the other additives such as surface-active agents may also be prepared by inicronisation, for instance in view to obtain them in the form of microspheres usually having a diameter of about 1 to 10 gm, namely for the manufacture of microcapsules for controlled or sustained release of the active ingredients.

Suitable surface-active agents, also known as emulgent or emulsifier, to be used in the pharmaceutical compositions of the present invention are non-ionic, cationic and/or anionic materials having good emulsifying, dispersing and/or wetting properties. Suitable anionic surfactants include both water-soluble soaps and water-soluble synthetic surface-active agents. Suitable soaps are alkaline or alkaline-earth metal salts, unsubstituted or substituted ammonium salts of higher fatty acids (C_{10}-C_{22}), e.g. the sodium or potassium salts of oleic or stearic acid, or of natural fatty acid mixtures obtainable form coconut oil or tallow oil. Synthetic surfactants include sodium or calcium salts of polyacrylic acids; fatty sulphonates and sulphates; sulphonated benzimidazole derivatives and alkylarylsulphonates. Fatty sulphonates or sulphates are usually in the form of alkaline or alkaline-earth metal salts, unsubstituted ammonium salts or ammonium salts substituted with an alkyl or acyl radical having from 8 to 22 carbon atoms, e.g. the sodium or calcium salt of lignosulphonic acid or dodecylsulphonic acid or a mixture of fatty alcohol sulphates obtained from natural fatty acids, alkaline or alkaline-earth metal salts of sulphuric or sulphonic acid esters (such as sodium lauryl sulphate) and sulphonic acids of fatty alcohol/ethylene oxide adducts. Suitable sulphonated benzimidazole derivatives preferably contain 8 to 22 carbon atoms. Examples of alkylarylsulphonates are the sodium, calcium or alcanolamine salts of dodecylbenzene sulphonic acid or dibutyl-naphtalenesulphonic acid or a naphtalene-sulphonic acid/formaldehyde condensation

product. Also suitable are the corresponding phosphates, e.g. salts of phosphoric acid ester and an adduct of p-nonylphenol with ethylene and/or propylene oxide, or phospholipids. Suitable phospholipids for this purpose are the natural (originating from animal or plant cells) or synthetic phospholipids of the cephalin or lecithin type such as e.g. phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerine, lysolecithin, cardiolipin, dioctanylphosphatidyl-choline, dipalmitoylphosbatidyl -choline and their mixtures.

Suitable non-ionic surfactants include polyethoxylated and polypropoxylated derivatives of alkylphenols, fatty alcohols, fatty acids, aliphatic amines or amides containing at least 12 carbon atoms in the molecule, alkylarenesulphonates and dialkylsulphosuccinates, such as polyglycol ether derivatives of aliphatic and cycloaliphatic alcohols, saturated and unsaturated fatty acids and alkylphenols, said derivatives preferably containing 3 to 10 glycol ether groups and 8 to 20 carbon atoms in the (aliphatic) hydrocarbon moiety and 6 to 18 carbon atoms in the alkyl moiety of the alkylphenol. Further suitable non-ionic surfactants are water-soluble adducts of polyethylene oxide with poylypropylene glycol, ethylenediaminopolypropylene glycol containing 1 to 10 carbon atoms in the alkyl chain, which adducts contain 20 to 250 ethyleneglycol ether groups and/or 10 to 100 propyleneglycol ether groups. Such compounds usually contain from 1 to 5 ethyleneglycol units per propyleneglycol unit. Representative examples of non-ionic surfactants are nonylphenol -polyethoxyethanol, castor oil polyglycolic ethers, polypropylene/polyethylene oxide adducts, tributylphenoxypolyethoxyethanol, polyethyleneglycol and octylphenoxypolyethoxyethanol. Fatty acid esters of polyethylene sorbitan (such as polyoxyethylene sorbitan trioleate), glycerol, sorbitan, sucrose and pentaerythritol are also suitable non-ionic surfactants.

Suitable cationic surfactants include quaternary ammonium salts, particularly halides, having 4 hydrocarbon radicals optionally substituted with halo, phenyl, substituted phenyl or hydroxy; for instance quaternary ammonium salts containing as N-substituent at least one C8C22 alkyl radical (e.g. cetyl, lauryl, palmityl, myristyl, oleyl and the like) and, as further substituents, unsubstituted or halogenated lower alkyl, benzyl and/or hydroxy-lower alkyl radicals.

A more detailed description of surface-active agents suitable for this purpose may be found for instance in "McCutcheon's Detergents and Emulsifiers Annual" (MC Publishing Crop., Ridgewood, New Jersey, 1981), "Tensid-Taschenbuew', 2 d ed. (Hanser Verlag, Vienna, 1981) and "Encyclopaedia of Surfactants, (Chemical Publishing Co., New York, 1981).

Compounds of the invention and their physiologically acceptable salts (hereafter collectively referred to as the active ingredients) may be administered by any route appropriate to the condition to be treated, suitable routes including oral, rectal, nasal, topical (including ocular, buccal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural). The preferred route of administration may vary with for example the condition of the recipient.

While it is possible for the active ingredients to be administered alone it is preferable to present them as pharmaceutical formulations. The formulations, both for veterinary and for human use, of the present invention comprise at least one active ingredient, as above described, together with one or more pharmaceutically acceptable carriers therefore and optionally other therapeutic ingredients. The carrier(s) optimally are "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof. The formulations include those suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined

amount of the active ingredient; as a powder or granules; as solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein. For infections of the eye or other external tissues e.g. mouth and skin, the formulations are optionally applied as a topical ointment or cream containing the active ingredient(s) in an amount of, for example, 0.075 to 20% w/w (including active ingredient(s) in a range between 0.1% and 20% in increments of 0.1% w/w such as 0.6% w/w, 0.7% w/w, etc), preferably 0.2 to 15% w/w and most preferably 0.5 to 10% w/w. When formulated in an ointment, the active ingredients may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredients may be formulated in a cream with an oil-in-water cream base. If desired, the aqueous phase of the cream base may include, for example, at least 30% w/w of a polyhydric alcohol, i.e. an alcohol having two or more hydroxyl groups such as propylene glycol, butane 1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol (including PEG400) and mixtures thereof. The topical formulations may desirably include a compound which enhances absorption or penetration of the active ingredient through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethylsulfoxide and related analogs.

The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While the phase may comprise merely an emulsifier (otherwise known as an emulgent), it desirably comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Optionally, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is

also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make up the so-called emulsifying wax, and the wax together with the oil and fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties, since the solubility of the active compound in most oils likely to be used in pharmaceutical emulsion formulations is very low. Thus the cream should optionally be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Formulations suitable for topical administration to the eye also include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the active ingredient. The active ingredient is optionally present in such formulations in a concentration of 0.5 to 20%, advantageously 0.5 to 10% particularly about 1.5% w/w. Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate. Formulations suitable for nasal administration wherein the carrier is a solid include a coarse powder having a particle size for example in the range 20 to 500 microns (including particle sizes in a range between 20 and 500 microns in increments of 5 microns such as 30 microns, 35 microns, etc), which is administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nasal passage from a container of the powder held close up to the nose.

Suitable formulations wherein the carrier is a liquid, for administration as for example a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient. Formulations suitable for aerosol administration may be prepared according to conventional methods and may be delivered with other therapeutic agents.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or unit daily sub-dose, as herein above recited, or an appropriate fraction thereof, of an active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavoring agents.

Compounds of the invention can be used to provide controlled release pharmaceutical formulations containing as active ingredient one or more compounds of the invention ("controlled release formulations") in which the release of the active ingredient can be controlled and regulated to allow less frequency dosing or to improve the pharmacokinetic or toxicity profile of a given invention compound. Controlled release formulations adapted for oral administration in which discrete units comprising one or more compounds of the invention can be prepared according to conventional methods.

Additional ingredients may be included in order to control the duration of action of the active ingredient in the composition. Control release compositions may thus be achieved by selecting appropriate polymer carriers such as for example polyesters, polyamino acids, polyvinyl pyrrolidone, ethylene-vinyl acetate copolymers, methylcellulose, carboxymethylcellulose, protamine sulfate and the like. The rate of drug release and duration of action may also be controlled by incorporating the active ingredient into particles, e.g. microcapsules, of a polymeric substance such as hydrogels, polylactic acid, hydroxymethylcellulose, polyniethyl methacrylate and the other above-described polymers. Such methods include colloid drug delivery systems like liposomes, microspheres, microemulsions, nanoparticles, nanocapsules and so on. Depending on the route of administration, the pharmaceutical composition may require protective coatings. Pharmaceutical forms suitable for injectionable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation thereof. Typical carriers for this purpose therefore include biocompatible aqueous buffers, ethanol, glycerol, propylene glycol, polyethylene glycol and the like and mixtures thereof.

In view of the fact that, when several active ingredients are used in combination, they do not necessarily bring out their joint therapeutic effect directly at the same time in the mammal to be treated, the corresponding composition may also be in the form of a medical kit or package containing the two ingredients in separate but adjacent repositories or compartments. In the latter context, each active ingredient may therefore be formulated in a way suitable for an administration route different from that of the other ingredient, e.g. one of them may be in the form of an oral or parenteral formulation whereas the other is in the form of an ampoule for intravenous injection or an aerosol.

The compounds according to the formulas of the application like (I), (II), (III) can be prepared while using a series of chemical reactions known to those skilled in the art, altogether making up the process for preparing said compounds and exemplified further. The processes described further are only meant as examples and by no means are meant to limit the scope of the present invention.

Examples

Example1: General methods for Antiviral screening

Anti-HIV assay: The inhibitory activity of compounds of the invention can be tested for their potential to inhibit the replication of HIV and SIV in a cell culture model for acute infection. Compounds can be tested against HIV-1 strains (HE, NL43, MN, III_B), HIV-2 strains (ROD, EHO, RF), and SIV (MAC251) for inhibition of virus-induced cytopathicity in MT-4 cells (or CEM or C8166 or Molt4/C8 cells), using the colorimetric test described by Pauwels et al. in *J. Virol. Methods* (1988) 20:309-321 or a microscopic investigation of the cytopathogenic effect, evaluation being made 4 to 5 days post-infection. For example microtiter 96-well plates containing ~ 3 x 10^5 CEM cells/ml, infected with 100 $CCID_{50}$ of HIV per ml and containing appropriate dilutions of the test compounds can be used.

A rapid and automated assay procedure can be used for the in vitro evaluation of anti-HIV agents. An HTLV-1 transformed T4-cell line MT-4, which was previously shown to be highly susceptible to and permissive for HIV infection, can serve as the target cell line. Inhibition of the HIV-induced cytopathogenic effect is used as the end point. The viability of both HIV- and mock-infected cells is also assessed spectrophotometrically via in situ reduction of 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Methods comprise for example the microscopic examination of CEM, C8166 or Molt4/C8 giant (syncytium) cell formation, after 4 to 5 days of incubation at 37°C in a CO_2-controlled humidified atmosphere. The 50 % cytotoxic concentration (CC_{50} in µg/ml) is defined as the concentration of compound that reduces the absorbance of the mock-infected control sample by 50 %. The percent protection achieved by the compound in HIV-infected cells is calculated by the following formula:

$$\frac{(OD_T)_{HIV} - (OD_C)_{HIV}}{(OD_C)_{MOCK} - (OD_C)_{HIV}} \quad \text{expressed in \%}$$

whereby $(OD_T)_{HIV}$ is the optical density measured with a given concentration of the test compound in HIV-infected cells; $(OD_C)_{HIV}$ is the optical density measured for the control untreated HIV-infected cells; $(OD_C)_{MOCK}$ is the optical density measured for the control untreated mock-infected cells; all optical density values are determined at 540 nm. The dose achieving 50 % protection according to the above formula is defined as the 50 % inhibitory concentration (IC_{50} in µg/ml). The ratio of CC_{50} to IC_{50} is defined as the selectivity index (SI).

Cells: MT-4 cells (Miyoshi *et al.*, 1982) are grown and maintained in RPMI 1640 medium supplemented with 10 % heat-inactivated fetal calf serum, 2 mM l-glutamine, 0.1 % sodium bicarbonate, and 20 µg of gentamicin per ml.

Viruses: The HIV-1 (IIIB, NL4.3) strain (Adachi *et al.*, 1986) is a molecular clone obtained from the National Institutes of Health (Bethesda, MD). The HIV-1 strain SO561945 is a strain resistant to non-nucleoside reverse transcriptase inhibitors. The HIV-2 (ROD, EHO) (Barr-Sinoussi et al., 1983) stock is obtained from culture supernatant of HIV-2 infected cell lines. Mac251 is a SIV strain.

References:

Adachi, A., Gendelman, H., Koenig, S., Folks, T;, Willey, R., Rabson, A. and Martin, M (1986) Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone, *J. Virol.*, 59, 284-291. Barr-Sinoussi, F., Chermann, J.C., Rey, F., Nugeyre, M.T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., V, Zinet-Brun, F., Rouzioux, C., Rozenbaum, W., Montagnier, L. (1983).

Isolation of a T-lyphotropic retrovirus from patient at risk for AIDS, *Science* (Wash DC) *220*, 868-871. Miyoshi, I., Taguchi, H., Kobonishi, I., Yoshimoto, S., Ohtsuki, Y., Shiraishi, Y., and Akagi, T. (1982) Type C virus-producing cell lines derived from adult T cell leukemia *Gann mongr. 28*, 219-228.

Cytostatic activity assays: All assays are performed in 96-well microtiter plates. To each well are added 5 – 7.5 x 10^4 cells and a given amount of the test compound. The cells are allowed to proliferate for 48 h (murine leukemia L1210) or 72 h (human lymphocyte CEM and Molt4/clone 8) at 37°C in a humidified CO_2-controlled atmosphere. At the end of the incubation period, the cells can be counted in a Coulter counter. The IC_{50} (50% inhibitory concentration) was defined as the concentration of the compound that reduced the number of cells by 50%.

Anti-BVDV assay:

Cells and viruses: Madin-Darby Bovine Kidney (MDBK) cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with BVDV-free 5% fetal calf serum (DMEM-FCS) at 37°C in a humidified, 5% CO_2 atmosphere. BVDV-1 (strain PE515) was used to assess the antiviral activity in MDBK cells. Vero cells (ATCC CCL81) were maintained in MEM medium supplemented with 10% inactivated calf serum, 1% L-glutamine and 0.3% bicarbonate.

Anti-BVDV assay: Ninety-six-well cell culture plates were seeded with MDBK cells in DMEM-FCS so that cells reached 24 hr later confluency. Then medium was removed and serial 5-fold dilutions of the test compounds were added in a total volume of 100 ul, after which the virus inoculum (100 ul) was added to each well. The virus inoculum used resulted in a greater than 90% destruction of the cell monolayer after 5 days incubation at 37°C. Uninfected cells and cells receiving virus without compound were included in each assay plate. After 5 days, medium was removed and 90 µl of DMEM-FCS and 10 ul of MTS/PMS solution (Promega) was added to each well. Following a 2 hr incubation period at 37°C the optical density of the wells was read at 498 nm in a microplate reader. The 50% effective concentration (EC_{50}) value was defined as the concentration of compound that protects 50% of the cell monolayer from virus-induced cytopathic effect.

Anti-HCV assay/ Replicon assay: Huh-5-2 cells [a cell line with a persistent HCV replicon I389luc-ubi-neo/NS3-3'/5.1; replicon with firefly luciferase-ubiquitin-neomycin phosphotransferase fusion protein and EMCV-IRES driven NS3-5B HCV polyprotein] van be cultured in RPMI medium (Gibco) supplemented with 10% fetal calf serum, 2mM

L-glutamine (Life Technologies), 1x non-essential amino acids (Life Technologies); 100 IU/ml penicillin and 100 ug/ml streptomycin and 250 ug/ml G418 (Geneticin, Life Technologies). Cells can be seeded at a different densities, particularly in a density of 7000 cells per well in 96 well View Plate™ (Packard) in medium containing the same components as described above, except for G418. Cells than can be allowed to adhere and proliferate for 24 hr. At that time, culture medium can be removed and serial dilutions of the test compounds can be added in culture medium lacking G418. Interferon alfa 2a (500 IU) can be included as a positive control. Plates can further be incubated at 37°C and 5% CO_2 for 72 hours. Replication of the HCV replicon in Huh-5 cells results in luciferase activity in the cells. Luciferase activity is measured by adding 50 µl of 1 x Glo-lysis buffer (Promega) for 15 minutes followed by 50 µl of the Steady-Glo Luciferase assay reagent (Promega). Luciferase activity can be measured with a luminometer and the signal in each individual well is expressed as a percentage of the untreated cultures. Parallel cultures of Huh-5-2 cells, seeded at a density of 7000 cells/ well of classical 96-well cel culture plates (Becton-Dickinson) can be treated in a similar fashion except that no Glo-lysis buffer or Steady-Glo Luciferase reagent is added. Instead the density of the culture can be measured by means of the MTS method (Promega).

Anti-Coxsackie virus assay: Ninety-six-well cell culture plates can be seeded with Vero cells in DMEM medium containing 10 fetal calf serum (FCS) so that cells reache confluency 24 -48 hr later. Medium can then be removed and serial 5-fold dilutions of the test compounds can be added in a total volume of 100 ul, after which the virus inoculum (100 µl) can be added to each well. The virus inoculum used results normally in a 90 – 100 % destruction of the cell monolayer after 5 days incubation at 37°C. Uninfected cells and cells receiving virus without compound can be included in each asay plate. After 5 days, the medium can be removed and 90 µl of DMEM-FCS and 10 µl of MTS/PMS solution (Promega) was added to each well. Following a 2 h incubation period at 37°C, the optical density of the wells can be read at 498 nm in a microplate reader. The 50% effective concentration (EC50) value can than be defined as the concentration of compound that protects 50% of the cell monolayer from virus-induced cytopathic effect.

Anti-Herpes simplex virus, varicella-zoster virus and cytomegalovirus assays: The antiviral assays HSV-1, HSV-2, VZV, CMV were based on inhibition of virus-induced cytopathicity in HEL cell cultures. Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the virus dose required to infect 50% of the cell cultures. After a 1- to 2-h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying compound concentrations of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds.

Feline corona virus assay: Feline Crandel kidney cells were seeded in 96-well microtiter plates at 24,000 cells/well. Then, 24 hrs later, an appropriate inoculum of FCV is added together with 5-fold dilutions of the test compounds. After 4 days, a MTS/PMS solution was added to each well. Following a 90 min incubation period at 37°C, the optical density of the wells was read at 498 nm in a microplate reader.

SARS virus assay: Vero cells were seeded in 96-well microtiter plates and grown till confluency. Then, an appropriate inoculum of SARS virus able to kill the cell culture (cytopathicity) within 72 hrs is added together with 5-fold dilutions of the test compounds. After 3 days, a MTS/PMS solution was added to each well. Following a 3 hr incubation period at 37°C the optical density of the wells was read at 498 nm in a microplate reader.

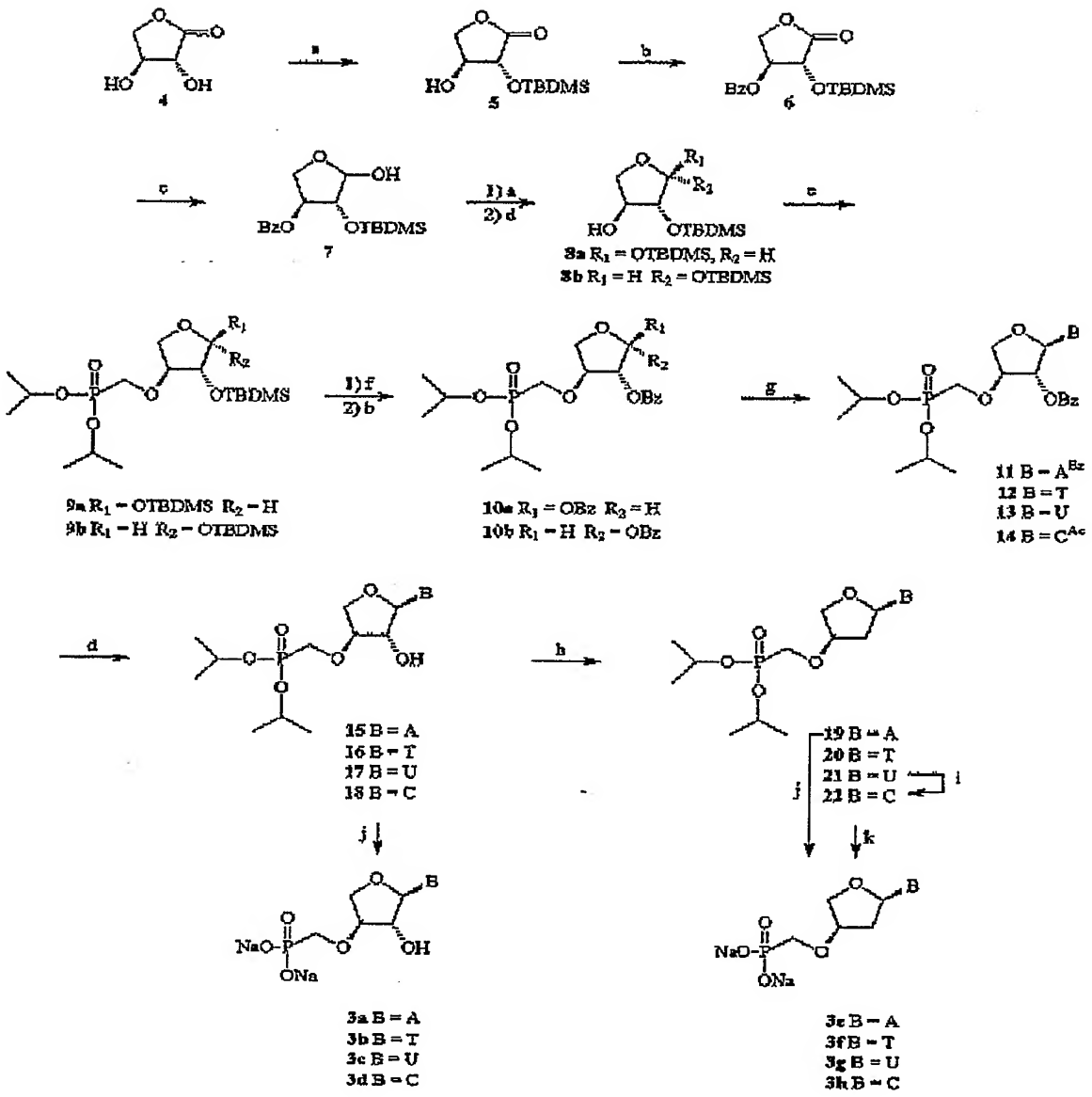
Example 2: Materials and general preparation methods

For all reactions, analytical grade solvents were used. All moisture sensitive reactions were carried out in oven-dried glassware (135 °C) under a nitrogen atmosphere. Anhydrous THF was refluxed over sodium/benzophenone and distilled. A Varian Unity 500 MHz spectrometer and a 200 MHz Varian Gemini apparatus were used for ^{1}H NMR and ^{13}C NMR. Exact mass measurements were performed on a quadrupole time-of-flight mass spectrometer (Q-Tof-2, Micromass, Manchester, UK) equipped with a standard electrospray-ionization (ESI) interface; samples were infused in i-PrOH/H_2O 1:1 at 3

μL/min. Precoated aluminum sheets (Fluka Silica gel/TLC-cards, 254nm) were used for TLC; The spots were examined with UV light. Column chromatography was performed on ICN silica gel 63-200 60Å.

The nucleosides (**3** a-h) were synthesized starting from (R,R)-2,3-dihydroxy-dihydro-furan-1-one (4) (Scheme 1).[13] The hydroxyl group in position 2 can be selectively protected with a TBDMS group. The free hydroxyl group of **5** is then protected by benzoylation and the lacton is reduced to the hemiketal using Dibal-H in THF. The anomeric hydroxyl group is protected with a TBDMS group and the O-benzoyl group is removed with ammonia in methanol. At the stage of **8**, the phosphonate function is introduced using the triflate of diisopropylphosphonomethyl alcohol and NaH in THF. The two silyl protecting groups of **9** are removed and replaced by benzoyl protecting groups. The presence of a 2-O-benzoyl group allows selective introduction of the base moiety in the β-configuration. The nucleobases (uracil, thymine, N^6-benzoyladenine, N^4-acetylcytosine) are introduced after silylation and using $SnCl_4$ as Lewis catalyst. Deprotection of **11-14** is done in two steps, first, removal of the benzoyl protecting groups with ammonia in methanol (yielding **15-18**), and, second, hydrolysis of the diisopropyl protecting groups with TMSBr at room temperature (giving **3** a-d). In order to obtain the 2'-deoxygenated analogues, the 2'-OH group of **15-17** is removed by Barton deoxygenation,[16,17] giving **19-21**. Compound **22** is obtained from **21**. Hydrolysis of the phosphonate ester function of **19** was carried out with TMSBr at room temperature. However, for the compounds **20-22**, TMSBr rapidly cleaved the nucleobase from the sugar even at 0°C. For this reason, TMSI was used for hydrolysis of the (diisopropylphosphono)-methyl group of **20-22**. After purification by silica gel chromatography, sephadex-DEAE A-25 resin and Dowex-sodium ion exchange resin, nucleoside phosphonates acid **3** e-h were obtained.

Scheme 1: general scheme representing the method of preparation



Conditions: a) TBDMSCl, imidazole, MeCN b) BzCl, pyridine c) Dibal-H, THF d) Sat. NH_3 in MeOH e) Trifluoromethanesulfonate of diisopropylphosphonylmethanol, NaH, THF f) TFA/H_2O g) $SnCl_4$, MeCN h) 1. $\phi OC(S)Cl$, DMAP, MeCN 2.Bu_3SnH, AIBN i) $P(O)Cl_3$, 1,2,4-triazole, DCM 2. j) 1. TMSBr, DCM sephadex-DEAE, Dowex- Na^+ k) 1. TMSI, DCM 2. sephadex-DEAE, Dowex- Na^+

Example 3: Preparation of Intermediate compounds

2-O-tributyldimethylsilyl-L-threonolactone (5)

To the solution of (3R,4S)-dihydro-3,4-dihydroxyfuran-2(3H)-one 4 (10.8 g, 92 mmol) and imidazole (12.5 g, 184 mmol) in 250 mL MeCN was added TBDMSCl (31.2 g, 3.17mmol) at 0 °C in one portion. The reaction mixture was slowly warmed to room temperature and stirred overnight. The reaction mixture was concentrated. The residue was partitioned between H_2O and EtOAc. The organic layer was washed with water and brine, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (n-hexane/EtOAC=6:1) to afford 5 (15.2 g, 65.4 mmol, 71 %) as a colorless solid. ^{1}H NMR (200 MHz, DMSO-d6) δ_H 0.12 (s, 6H, $SiCH_3$), 0.90 (s, 9H, CH_3), 3.86 (dd, J_1 = 6.96 Hz, J_2 = 7.70 Hz, 1H, C(4')H_a), 4.11- 4.36 (m, 3H, OH, C(3')H, C(4')H_b), 5.82 (d, J = 5.13 Hz, 1H, C(2')H); ^{13}C NMR (200 MHz, DMSO-d6) δ_C -4.93($SiCH_3$), 17.99 ($\underline{C}(CH_3)_3$), 25.61($C(\underline{C}H_3)_3$), 69.62(C-4'), 72.62(C-2'), 74.59(C-3'), 174.60(C-1'); Exact mass calcd for $C_{10}H_{20}O_4Si_1Na_1$ $[M+Na]^+$ 255.1028 found 255.1010.

2-O-tributyldimethylsilyl-3-O-benzoyl-L-threonolactone (6)

To the solution of 5 (18.00 g, 77.5 mmol) in 200 mL pyridine was added dropwise BzCl (11.2 mL, 96.9 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was concentrated and coevaporated with 20 mL toluene two times *in vacuo*. The residue was partitioned between H_2O (100 mL) and EtOAc (350 mL). The organic layer was washed with water and brine, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (n-hexane/EtOAc=8:1) to afford 6 (25.9 g, 77.0 mmol) as a colorless solid in 99% yield. ^{1}H NMR (500 MHz, DMSO-d6) δ_H 0.14 (d, J_1 = 13.2, 6H, $SiCH_3$), 0.87 (s, 9H, CH_3), 4.23 (dd, J_1 = 6.8 Hz, J_2 = 9.3 Hz, 1H, C(4') H_a), 4.68 (dd, J_1 = 7.3 Hz, J_2 = 9.3 Hz, 1H, C(4') H_b), 4.96 (d, J = 6.8 Hz, 1H, C(2')H), 5.48 (dd, J_1 = 7.3 Hz, J_2 = 13.0 Hz, 1H, C(3')H), 7.57 –8.01 (m, 5H, Ar-H'); ^{13}C NMR (500 MHz, DMSO-d6) δ_c -5.16($Si\underline{C}H_3$), –4.84 ($Si\underline{C}H_3$), 17.84 ($\underline{C}(CH_3)_3$), 25.42($C(\underline{C}H_3)_3$), 67.20 (C-4'), 71.67(C-2'), 75.46(C-3'), 128.65 (aroma-C), 128.90(aroma-C), 129.36(aroma-C), .133.94(aroma-C), 165.08 (Bz-

CO), 172.76 (C-1'); Exact mass calcd for $C_{17}H_{25}O_5Si_1$ $[M+H]^+$ 337.1471 found 337.1465;

2-O-tributyldimethylsilyl-3-O-benzoyl-L-threose (7)

To the solution of **6** (10.0 g, 29.7 mmol) in 100 mL dry THF was slowly dropwise added 1.0 M diisopropyl aluminiumhydride (37.1 mL , 37.1 mmol) in toluene at -78 °C. The reaction mixture was stirred at -78 °C, and as soon as the starting material was completely consumed (TLC, 4-10 hours), methanol (10 mL) was added over a period of 5 min. to quench the reaction. The cooling bath was removed, 100mL of a sat. aq. sodium potassium tartrate solution and 200 mL of EtOAc were added and the mixture stirred vigorously for 3 hours. The organic layer was washed with water and brine, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (n-hexane/EtOAc=8:1) to afford 7 (7.40 g, 21.8 mmol) as a colorless solid in 73% yield. ^{1}H NMR (200 MHz, DMSO-d6) δ_H 0.10 (s, 6H, Si-CH_3), 0.87 (s, 9H, CH_3), 3.93 (dd, J_1 = 9.89 Hz, J_2 = 3.66 Hz, 1H, C(4')H_a), 4.16 (br s, 1H, OH), 4.24 (dd, J_1 = 10.26 Hz, J_2 = 5.86 Hz, 1H, C(4')H_b), 5.02-5.07 (m, 2H, C(2')H, C(3')H), 6.54 (d, J = 4.76 Hz, 1H, C(1')H), 7.51-8.00 (m, 5H, Ar-H); ^{13}C NMR (200 MHz, DMSO-d6) δ_C -7.39 ($SiCH_3$), -7.30 ($SiCH_3$), 15.41 (C(CH_3)$_3$), 23.27 (C(CH_3)$_3$), 67.06 (C-4')), 77.14 (C-2')), 78.87 (C-3'), 100.23 (C-1'), 126.58(aroma-C), 127.09 (aroma-C), 131.40 (aroma-C), 163.18 (Bz-CO); Exact mass calcd for $C_{17}H_{26}O_5Si_1Na_1$ $[M+Na]^+$ 361.1447 found 361.1452;

1α,2-di-O-tributyldimethylsilyl-L-threose (8a) and 1β,2-di-O-tributyldimethylsilyl-L-threose (8b)

To the solution of 7 (7.30 g, 21.6 mmol) and imidazole (2.94 g, 43.1 mmol) in 100 mL MeCN was added TBDMSCl (0.98 g, 23.8 mmol) at 0 °C in one portion. The reaction mixture was slowly warmed to room temperature and stirred overnight. The reaction mixture was concentrated. The residue was partitioned between H_2O and EtOAc. The organic layer was washed with water and brine, and concentrated in vacuo. The residue was dissolved in MeOH saturated with ammonia (100 mL), and the reaction mixture was stirred at room temperature overnight. The mixture was concentrated, and the residue was

purified by column chromatography (n-hexane:EtOAc, 20:1, 10:1) to give compound **8a** (2.22 g, 1.40 mmol) as colorless oil in 42% yield and **8b** (1.00g, 1.40 mmol) as colorless oil in 19% yield.

8a

^{1}H NMR (200 MHz, DMSO-d6) δ_H 0.06-0.08 (m, 12H, Si- CH_3), 0.87 (s, 18H, CH_3), 3.59-3.65 (m, C(2')H, 1H,), 3.87-3.99 (m, 3H, C4' H_a C(3')H, C(4') H_b), 5.00 (d, J = 1.1 Hz, C(1')H), 5.07-5.10 (m, 1H, OH); ^{13}C NMR (200 MHz, DMSO-d6) δ_C -5.14 (Si$\underline{C}H_3$), -4.92 (Si$\underline{C}H_3$), -4.65 (Si$\underline{C}H_3$), -4.38 (Si$\underline{C}H_3$), 17.66 (C($\underline{C}H_3$)$_3$), 17.81 (C($\underline{C}H_3$)$_3$), 25.61 (C($\underline{C}H_3$)$_3$), 25.73 (C($\underline{C}H_3$)$_3$), 71.92 (C-4'), 76.66 (C-2'), 85.58 (C-3'), 103.91 (C-1'); Exact mass calcd for $C_{16}H_{36}O_4Si_2Na_1$ 371.2050 found 371.2059.

8b

^{1}H NMR (200 MHz, DMSO-d6) δ_H 0.05 (s, 6H, $SiCH_3$), 0.06 (d, J_1 = 5.2 Hz, $SiCH_3$), 0.86 (s, 9H, CH_3), 0.87 (s, 9H, CH_3), 3.41 (dd, J_1 = 8.0 Hz, J_2 = 3.7 Hz , C(2')H); 3.81 (dd, J_1 = 5.2 Hz, J_2 = 3.7 Hz, C(3')H), 3.94-4.07 (m, 2H, C(4')H_a C(4')H_b), 5.12-5.15 (m, 2H, OH, C(1')H); ^{1}H NMR 200MHz (DMSO-d6 + 1D D_2O) δ_H 0.02(s, 6H, $SiCH_3$),0.04(d, J_2 = 4.4 Hz, $SiCH_3$), 0.84 (s, 18H, CH_3), 3.39(dd, J_1 = 7.7 Hz, J_2 = 3.6 Hz, C(2')H); 3.79 (dd, J_1 = 4.4 Hz, J_2 = 4.4 Hz, C(3')H), 3.92-4.07 (m, 2H, C(4')H_a C(4')H_b) 5.10 (d, 1H, J_2 = 3.6 Hz, C(1')H); ^{13}C NMR (200 MHz, DMSO-d6) δ_C -4.95 ($SiCH_3$), -4.74 ($SiCH_3$), -4.67 (Si$\underline{C}H_3$), 17.45 ($\underline{C}$(CH_3)$_3$), 25.64 (C($\underline{C}H_3$)$_3$), 25.79 (C($\underline{C}H_3$)$_3$), 70.80 (C-4'), 74.17 (C-2'), 79.45 (C-3'), 97.11 (C-1'); Exact mass calcd for $C_{16}H_{36}O_4Si_2Na_1$ 371.2050 found 371.2052.

1α,2-di-O-tributyldimethylsilyl-3-O-(diisopropylphosphonomethyl)-L-threose (9a) and 1β,2-di-O-tributyldimethylsilyl-3-O-(diisopropylphosphonomethyl)-L-threose (9b)

To a solution of **8a** (3.41 g, 9.8 mmol) in dried THF (25 mL) was added sodium hydride (80% dispersion in mineral oil 0.56 mg, 19.6 mmol) at -78°C. Then the solution of the triflate of diisopropylphosphonomethanol (5.80 g, 19.6 mmol) in dried THF (10 mL) was dropwise added, and the reaction mixture was slowly warmed to room temperature. The reaction was quenched with sat. $NaHCO_3$ and concentrated. The residue was partitioned between H_2O and EtOAc. The organic layer was washed with water and brine, and concentrated in vacuo. The residue was purified by chromatography on a silica gel

column (n-hexane/ EtOAC=2:1) to afford **9a** (4.75 g, 9.0 mmol, 92%) as colorless oil. ^{1}H NMR (200 MHz, DMSO-d6) δ_H 0.06-0.10 (m, 12H, $SiCH_3$), 0.86 (s, 9H, $C(CH_3)_3$), 0.87 (s, 9H, $C(CH_3)_3$), 1.22-1.26 (m, 12H, $C(CH_3)_2$), 3.75 (d, J = 9.2 Hz, 2H, CH_2), 3.78 (d, J = 9.2 Hz, 1H, C(4')H_a), 3.88-3.95 (m, 1H, C(3')H), 3.99 (s, 1H, C(2')H), 4.10 (dd, J_1 = 9.2 Hz, J_2 = 8.6 Hz, 1H, C(4')H_b), 4.52-4.68 (m, 2H, CH), 5.02(s, 1H, C(1')H); Exact mass for $C_{23}H_{52}O_7P_1Si_2$ $[M+H]^+$ Calcd. 527.2989 found 527.2988.

The synthesis of **9b** started from **8b** (2.00 g, 5.7 mmol) and followed the same procedure as for the synthesis of **9a**, from **8a** (2.7 g, 5.1 mmol, 90%) as colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 0.08-0.11 (m, 12H, Si CH_3), 0.93 (br s, 18H, $C(CH_3)_3$), 1.33 (d, J = 6.2 Hz, 12H, $C(CH_3)_2$), 3.66-3.94 (m, 3H, C(4')H, PCH_2), 4.02-4.22 (m, 3H, C(2')H, C(3')H, C(4')H_b), 4.67-4.83 (m, 2H, $CH(CH_3)_2$), 5.13 (d, J = 3.7 Hz, 1H, C(1')H); ^{1}H NMR (200 MHz, DMSO-d6) δ_H 0.06-0.93 (m, 12H, $SiCH_3$), 0.87 (s, 18H, $C(CH_3)_3$), 1.22-1.26 (m, 12H, $C(CH_3)_2$), 3.58-3.65 (m, 1H, C(4') H_a), 3.78 (d, J = 9.2 Hz, PCH_2), 3.96-4.08 (m, 3H, C(2')H, C(3')H, C(4') H_b), 4.51-4.67 (m, 2H, $CH(CH_3)_2$), 5.15 (d, J = 3.7 Hz, 1H, C(1')H); ^{13}C NMR (200 MHz, DMSO-d6) δ_C -5.22 ($SiCH_3$), -5.07 ($SiCH_3$), -4.58 ($SiCH_3$), 17.88 ($C(CH_3)_3$),, 23.98 ($OCH(CH_3)_2$), 25.62 ($C(CH_3)_3$), 25.71 ($C(CH_3)_3$), 65.12 (d, $J_{P,C}$ = 173.6Hz, PCH2), 68.38 (C-4')), 70.87 ($OCH(CH_3)2$), 70.96 ($OCH(CH_3)_2$), 78.88 (C-2'), 85.68 (d, $J_{P,C}$ = 12.0 Hz, C-3'), 97.3 (C-1'); Exact mass calcd for $C_{23}H_{52}O_7P_1Si_2$ $[M+H]^+$ 527.2989 found 527.2972.

1α,2-O-benzoyl-3-O-(diisopropylphosphonomethyl)-L-threose (10a) and 1β,2-O-benzoyl-3-O-(diisopropylphosphonomethyl)-L-threose (10b)

A solution of **9a** (4.25 g, 8.1 mmol) in TFA-H_2O (3:1, 20 mL) was allowed to stand at room temperature for 2 h. The reaction mixture was neutralized with saturated $NaHCO_3$ solution. Then the mixture was partitioned between the DCM (400 mL) and water (20 mL). The organic layer was washed with water and brine, dried over $MgSO_4$, and then concentrated in vacuo. The residue was purified by chromatography on silica gel (DCM:MeOH=20:1) to give 3-O-diisopropylphosphonomethyl-L-threose (2.20 g, 7.3 mmol) as a colorless amorphous solid in 92% yield.

To the solution of 3-O-(diisopropylphosphonomethyl)-L-threose (687 mg, 2.3 mmol) in 100 mL pyridine was added dropwise BzCl (0.67 , 5.8 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was concentrated and coevaporated with 20 mL toluene two times in vacuo. The residue was partitioned between H_2O (20 mL) and EtOAc (150 mL). The organic layer was washed with water and brine, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (n-hexane/EtOAc=1:1) to afford **10a** and **10b** (1.0 g, 2.0 mmol) as colorless oil in 87% yield.

10a

^{1}H NMR (200MHz, DMSO-d6) δ_H 1.20-1.26 (m, 12H, $C(CH_3)_2$), 3.40-4.11 (m, 3H, PCH_2, C(4')H_a), 4.40-4.54 (m, 2H, C(3')H, C(4')H_b), 4.56-4.71 (m, 2H, $OCH(CH_3)_2$), 5.51 (s, 1H, C(2')H), 6.47 (s, 1H, C(1')H), 7.43-8.07 (m, 10H, Ar-H); ^{13}C NMR (200MHz, DMSO-d6) δ_C 23.82 (CH_3), 64.45 (d, J = 155.4 Hz, PCH_2), 70.59 (CH(CH_3)), 73.23 (C-4'), 80.12 (C-2'), 80.30 (C-2'), 99.78 (C-1'), 129.04(aroma-C), 129.83(aroma-C), 134.14 (aroma-C), 164.61 (Bz-CO), 165.07 (Bz-CO); Exact mass calcd for $C_{25}H_{31}O_9P_1Na_1$ $[M+Na]^+$ 529.1603 found 529.1601.

Example 4: Preparation of final compounds

1-(N^6-benzoyladenin-9-yl)-2-O-benzoyl-3-O-(diisopropylphosphonomethyl)-L-threose (11)

To a mixture of 10a (425 mg, 0.83 mmol) and silylated N^6-benzoyladenine (401 mg, 1.6 mmol) in dry MeCN (30 mL) was dropwise added $SnCl_4$ (0.3 mL, 2.5 mmol) under N_2 at room temperature The reaction mixture was stirred at r.t for 4-5 hours. Then the reaction was quenched with sat. $NaHCO_3$, and concentrated. The residue was partitioned between H_2O (20 mL)and EtOAc (100 mL). The organic layer was washed with water and brine, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (DCM/MeOH=40:1) to afford **11** (431mg, 0.69 mmol) as a colorless amorphous solid in 83% yield. ^{1}H NMR (500MHz, $CDCl_3$) δ_H 1.31-1.36 (m, 12H, CH_3), 3.94 (dd, J_1 = 14.0 Hz, J_2= 8.6 Hz, 1H, PC H_a), 4.01 (dd, J_1= 14.0 Hz, J_2= 8.6 Hz, 1H, PC H_b), 4.38 (dd, J_1= 11.0 Hz, J_2= 4.6 Hz, 1H, C(4')H_a), 4.50-4.52 (m, 2H, C(3')H, C(4') H_b), 4.73-4.80 (m, 2H, OCH), 5.08 (s, 1H, C(2')H), 6.56 (s, 1H, C(1')H), 7.48-7.65 (m, 6H, Ar-H),

8.02-8.08 (m, 4H, Ar-H), 8.50 (s,1H, Adinie-C(8)-H), 8.82 (s, 1H, Adinie-C(2)-H), 9.07 (br s, 1H, NH); ^{13}C NMR (500MHz, $CDCl_3$) δ_C 23.97 (CH_3), 24.01 (CH_3), 24.03 (CH_3), 24.06 (CH_3), 65.36 ($J_{P,C}$ = 168.9 Hz, PCH_2), 71.45 (POCH), 71.51(POCH), 73.55(C-4')), 80.27 (C-2')), 83.74 ($J_{P,C}$ = 9.8Hz, C-3'), 87.86 (C-1')), 122.72 (A-C(5)), 127.80 (aroma-C), 128.65 (aroma-C), 128.67 (aroma-C), 128.86 (aroma-C), 129.93 (aroma-C), 132.31(aroma-C), 133.99 (aroma-C), 141.98 (A-C(8)), 149.45 (A-C(6),151.59 (A-C(4)), 152.93 (A-C(2)), 164.44 (OBz(CO)), 165.17 (NBz(CO)); Exact mass calcd for $C_{30}H_{35}N_5O_8P_1$ $[M+H]^+$ 624.2223 found 624.2222.

1-(thymin-1-yl)-2-O-benzoyl-3-O-(diisopropylphosphonomethyl)-L-threose (12)

Thymine (0.34 g, 2.7 mmol), ammonia sulfate (10 mg, 0.07mmol) and 6 mL of HMDS were added to dried flask. The mixture was refluxed overnight under nitrogen. HDMS was removed in vacuo. To the flask with residue was added the solution of compound **10a** (0.92 g, 1.8 mmol) in 10 mL of dry MeCN followed by dropwise addition of $SnCl_4$ (640 µL 5.4 mmol) under N_2 at room temperature The reaction mixture was stirred for 4 hours. The reaction was quenched with sat. aq. $NaHCO_3$ and concentrated to a small volume. The residue was partitioned between H_2O (30 mL) and EtOAc (150 mL). The organic layer was washed with water and brine, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (n-hexane/EtOAc=1:1) to afford **12** (0.76 g, 1.4 mmol) as a colorless amorphous solid in 78% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.35 (d, J = 6.2 Hz, 12H, CH_3), 1.99 (d, J = 1.5 Hz, 3H, T-CH_3), 3.86-4.05 (m, 2H, PCH_2), 4.11-4.16 (m, 1H, C(4')H_a), 4.26 (br t, 1H, C(3')H), 4.40 (d, J = 10.6 Hz, 1H, C(4') H_b), 4.70-4.86 (m, 2H, $OCH(CH_3)_2$), 5.38 (s, 1H, C(2')H), 6.29 (t, J = 2.2 Hz, 1H, C(1')H), 7.43-7.66 (m, 4H, Ar-H, T-C(6)H), 8.02-8.07 (m, 2H, Ar-H), 9.13 (s, 1H, NH); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 12.42 (T-CH3), 23.83 ($CH(\underline{C}H_3)_3$), 23.92 ($CH(\underline{C}H_3)_3$), 64.48 (d, $J_{P,C}$ = 168.5 Hz, PCH_2), 71.29 ($\underline{C}H(CH_3)_3$), 71.45 ($\underline{C}H(CH_3)_3$), 72.72 (C-4'), 80.28 (C-2'), 83.70 ($J_{P,C}$ = 10.6 Hz, C-3'), 89.02 (C-1'), 111.39 (T-C(5)), 128.60 (aroma-C), 129.90 (aroma-C), 133.84 (T-C(6), 136.12 (aroma-C), 150.42 (T-C(2), 163.86 (T-C(4), 165.32 (Bz-CO); Exact mass calcd for $C_{23}H_{31}N_2O_9P_1$ $[M+H]^+$ 511.1845 found 511.1831.

1-(uracil-1-yl)-2-O-benzoyl-3-O-(diisopropylphosphonomethyl)-L-threose (13)

Uracil (0.81 g, 7.2 mmol), ammonia sulfate (10 mg, 0.07 mmol) and 20 mL of HMDS were added to dried flask. The mixture was refluxed overnight under nitrogen. HDMS was removed in vacuo. To the residue was added the solution of compound **10a** (2.43 g, 4.8 mmol) in 50 mL of dry MeCN followed by a dropwise addition of $SnCl_4$ (1.7 mL, 14.4 mmol). The reaction mixture was stirred for 4 hours. The reaction was quenched with sat. aq. $NaHCO_3$ and concentrated to small volume. The residue was partitioned between H_2O (30 mL) and EtOAc (100 mL). The organic layer was washed with water and brine, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (DCM/MeOH=25:1) to afford **13** (2.09 g, 4.2 mmol) as a colorless amorphous solid in 84%. ^1H NMR (500 MHz, DMSO-d6) δ_H 1.23-1.26 (m, 12H, CH_3), 3.97 (d, J= 9.0 Hz, 2H, PCH_2), 4.16 (dd, J_1= 10.7 Hz, J_2= 4.2 Hz, 1H, C(4')H_a), 4.36 (d, J = 10.7 Hz, 1H, C(4')H_b), 4.39-4.40 (m, 1H, C(3')H), 4.58-4.64 (m, 2H, $OCH(CH_3)_2$), 5.41 (s, 1H, C(2')H), 5.61 (d, J = 8.1 Hz, 1H, U-C(5)H), 6.02 (d, J = 2.0 Hz, 1H, C(1')H), 7.55-7.60 (m, 2H, Ar-H), 7.63 (d, J= 8.1 Hz, 1H, U-C(5)H), 7.70-7.73 (m, 1H, Ar-H), 8.02-8.04 (m, 2H, Ar-H), 11.4 (s, 1H, NH); ^{13}C NMR (500 MHz, DMSO-d6) δ_C 23.74 ($CH(\underline{C}H_3)_3$), 23.84 ($CH(\underline{C}H_3)_3$), 63.10 (d, $J_{P,C}$ = 168.5 Hz, PCH_2), 70.53 ($\underline{C}H(CH_3)_3$), 72.32 (C-4'), 79.83 (C-2'), 82.78 (C-3'), 89.06 (C-1'), 101.91 (U-C(5), 128.95 (aroma-C), 129.63 (aroma-C), 134.07 (aroma-C), 140.72 (U-C(6), 150.39 (U-C(2)), 163.19 (U-C(4), 164.73 (Bz-CO); Exact mass calcd for $C_{22}H_{29}N_2O_9P_1Na_1$ $[M+Na]^+$ 519.1508 found 519.1506.

1-(N^4-acetylcytosin-1-yl)-2-O-benzoyl-3-O-(diisopropylphosphonomethyl)-L-threose (14)

N^4-Acetylcytosine (0.41 g, 2.7 mmol) and ammonia sulfate (10 mg, 0.07 mmol) and 6 mL of HMDS were added to dried flask. The mixture was refluxed overnight under nitrogen. HDMS was removed in vacuo. To the residue was added the solution of compound **10a** (0.92 g, 1.8 mmol) in 10 mL of dry MeCN followed by a dropwise addition of stannic chloride (640 µL 5.4 mmol). The reaction mixture was stirred for 4 hours. The reaction was quenched with sat. aq. $NaHCO_3$ and concentrated to small volume. The residue was partitioned between H_2O (30 mL) and EtOAc (150 mL). The

organic layer was washed with water and brine, and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (n-hexane/EtOAc=2:1) to afford **14** (0.51 g, 0.94 mmol) as a colorless amorphous solid in 52% yield. ^{1}H NMR (200 MHz, DMSO-d6) δ_H 1.17-1.23 (m, 12H, CH(CH_3)$_2$), 2.10 (s, 3H, CH_3), 3.80-4.00 (m, 2H, PCH_2), 4.24-4.36 (m, 2H, C(4')H_a, C(3')H), 4.48-4.63 (m, 3H, C(4') H_b, OC$\underline{H}$(CH_3)$_2$), 5.44 (s, 1H, C(2')H), 6.04 (s, 1H, C(1')H), 7.27 (d, J = 7.7 Hz, 1H, C-C(5)H), 7.54-7.77 (m, 3H, Ar-H), 8.03-8.07 (m, 3H, Ar_o-H, C-C(6)H), 10.95 (s, 1H, NH); ^{13}C NMR (200 MHz, DMSO-d6) δ_C 23.76 (CH($\underline{C}H_3$)$_3$), 24.39 (Ac-CH_3), 63.77 (d, $J_{P,C}$ = 166.4 Hz, PCH_2), 70.44 ($\underline{C}$H(CH_3)$_3$), 70.59 ($\underline{C}$H(CH_3)$_3$), 73.56 (C-4'), 79.75 (C-3') 82.83 (d, $J_{P,C}$ = 13.7 Hz,C-3'), 90.74 (C-1'), 94.74 (C-C(5), 128.86 (aroma-C), 129.14 (aroma-C), 134.07 (aroma-C), 129.77 (aroma-C), 134.23 (aroma-C), 145.40 (C-C(6), 154.69 (C-C(2)), 162.95 (Bz-CO), 164.77 (C-C(4)); 171.26 (Ac-CO); Exact mass calcd for $C_{24}H_{33}N_3O_9P_1$ $[M+H]^+$ 538.1954 found 538.1956.

1-(adenin-9-yl)-3-O-(diisopropylphosphonomethyl)-L-threose (15)

A solution of **11** (431 mg, 0.80 mmol) in MeOH saturated with ammonia (100 mL) was stirred at room temperature overnight. The mixture was concentrated, and the residue was purified by column chromatography (CH_2Cl_2:MeOH=9:1) to give compound **15** (278 mg, 0.67 mmol) as a white powder in 84% yield. ^{1}H NMR (500 MHz, DMSO-d6): δ_H 1.21-1.26 (m, 12H, CH_3), 3.85-3.94 (m, 2H, P CH_2), 4.10-4.13 (m, 2H, C(4') H_a, C(3')H), 4.24-4.27 (m, 1H, C(4') H_b), 4.57-4.63 (m, 3H, C$\underline{H}$(CH)3, C(2')H), 5.93 (d, J = 2.1 Hz, 1H, C(1')H), 6.05 (br s, 1H, OH), 7.24 (s, 2H, NH2), 8.15 (s, 1H, C(2)H), 8.18 (s, 1H, C(8)H); ^{13}C NMR (200 MHz, DMSO-d6): δ_C 23.82 (CH_3), 63.5 ($J_{P,C}$ = 164.6 Hz, PCH_2), 70.41 (OCH), 70.53 (OCH), 71.65 (C-4'), 78.27 (C-2'), 85.62 ($J_{P,C}$ = 13.6 Hz, C-3'), 89.53 (C-1'), 118.79 (A-C(5), 139.39 (A-C(8)), 149.47 (A-C(6), 152.90 (A-C(4)), 156.24 (A-C(2)); Exact mass calcd for $C_{16}H_{27}N_5O_6P_1$ $[M+H]^+$ 416.1699 found 416.1681.

1-(thymin-1-yl)-3-O-(diisopropylphosphonomethyl)-L-threose (16)

A solution of **12** (715 mg, 1.7 mmol) in MeOH saturated with ammonia (100 mL) was stirred at room temperature overnight. The mixture was concentrated, and the residue was purified by column chromatography (CH_2Cl_2:MeOH=10:1) to give compound **16** (515

mg, 1.2 mmol) as a white powder in 71% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.27-1.33 (m, 12H, CH_3), 1.93 (d, J = 1.7 Hz, 3H, T- CH_3), 3.75 (d, J = 8.8 Hz, 2H, P CH_2), 4.13 (br t, 1H, C(3')H), 4.24-4.31 (m, 2H, C(4')H_2), 4.38 (s, 1H, C(2')H), 4.61-4.80 (m, 2H, OCH(CH_3)$_2$), 5.81 (s, 1H, C(1')H), 7.41 (d, J = 1.46 Hz, T-C(6)H), 10.27 (br s, 1H, NH); ^{13}C NMR (200 MHz, $CDCl_3$) δ_c 2.42 (T-CH3), 23.89 (CH($\underline{C}H_3$)$_3$), 64.46 (d, $J_{P,C}$ = 168.5 Hz, PCH_2), 71.26 ($\underline{C}$H(CH_3)$_3$), 73.54 (C-4'), 78.94 (C-2'), 85.33 (d, $J_{P,C}$ = 10.6 Hz, C-3'), 93.12 (C-1'), 110.12 (T-C(5)), 136.40 (T-C(6), 151.08 (T-C(2)), 164.56 (T-C(4)); Exact mass calcd for $C_{16}H_{28}N_2O_8P_1$ $[M+H]^+$ 407.1583 found 407.1568.

1-(uracil-1-yl)-3-O-(diisopropylphosphonomethyl)-L-threose (17)

A solution of **13** (2.03 g, 4.0 mmol) in MeOH saturated with ammonia (300 mL) was stirred at room temperature overnight. The mixture was concentrated, and the residue was purified by column chromatography (CH_2Cl_2:MeOH=20:1) to give compound **17** (1.52g, 3.8 mmol) as a white powder in 96% yield. ^{1}H NMR (200MHz, DMSO-d6): δ_H 1.19-1.25 (m, 12H, CH_3), 3.78 (dd, J_1 = 13.9 Hz, J_2 = 9.2 Hz, PCH_a) 3.85 (dd, J_1 = 13.9 Hz, J_2 = 9.2 Hz, PCH_b), 3.98-4.28 (m, 4H, C(2')H, C(3')H, C(4')H_2), 4.50- 4.66 (m, 2H, $\underline{C}$H(CH_3), 5.50 (d, J = 8.0 Hz, U-C(5)H), 5.66 (d, J = 1.5 Hz, OH), 5.93 (d, J = 4.4 Hz, C(1')H), 7.54(d, J = 8.0 Hz, U-C(6)H); ^{13}C NMR (200MHz, DMSO-d6) δ_c 23.70 (CH($\underline{C}H_3$)$_3$), 23.79 (CH($\underline{C}H_3$)$_3$), 63.29 (d, $J_{P,C}$ = 166.3 Hz, PCH_2), 70.34 ($\underline{C}$H(CH_3)$_3$), 70.47 ($\underline{C}$H(CH_3)$_3$), 72.29 (C-4'), 77.84 (C-2'), 85.23 ($J_{P,C}$ = 10.7 Hz, C-3'), 91.68 (C-1'), 101.12 (U-C(5)), 141.12 (U-C(6), 150.72 (U-C(2)), 163.46 (C-C(4)); Exact mass calcd for $C_{15}H_{26}N_2O_8P_1$ $[M+H]^+$ 393.1427 found 393.1425.

1-(cytosin-1-yl)-3-O-(diisopropylphosphonomethyl)-L-threose (18)

A solution of **14** (450 mg, 0.84 mmol) in MeOH saturated with ammonia (100 mL) was stirred at room temperature overnight. The mixture was concentrated, and the residue was purified by column chromatography (CH_2Cl_2:MeOH=20:1) to give compound **18** (281mg, 0.72 mmol) as a white powder in 86% yield. ^{1}H NMR (200MHz, DMSO-d6) δ_H 1.18-1.25 (m, 12H, CH_3), 3.72 (dd, J_1 = 13.6 Hz, J_2 = 8.8 Hz, PCH_a) 3.84 (dd, J_1 = 13.6 Hz, J_2 = 8.8 Hz, PCH_b), 3.95-4.05 (m, 3H, C(2')H, C(3')H, C(4')H_a), 4.25 (d, J = 9.5 Hz, C(4')H_b), 4.48- 4.64 (m, 2H, CH(CH_3), 5.65 (d, J = 7.6 Hz, C-C(5)H), 5.70(d, J = 1.5 Hz,

OH), 5.85(d, J= 15.4 Hz, C(1')H), 7.04 (br s, NH_a), 7.14(br s, NH_b), 7.50(d, J= 7.6 Hz, C-C(6)H); ^{13}C NMR (200MHz, DMSO-d6): δ_c 23.68 ($CH(\underline{C}H_3)_3$), 23.78 ($CH(\underline{C}H_3)_3$),64.46 (d, $J_{P,C}$ = 164.8 Hz, PCH_2), 70.30 ($\underline{C}H(CH_3)_3$), 70.42 ($\underline{C}H(CH_3)_3$), 72.00 (C-4'), 78.19 (C-2'), 85.66 (d, $J_{P,C}$ = 12.2 Hz, C-3'), 92.30 (C-1'), 93.46 (C-C(5)), 141.63 (C-C(6), 155.47 (C-C(2)), 165.94 (C-C(4)); Exact mass calcd for $C_{15}H_{27}N_3O_7P_1$ $[M+H]^+$ 392.1586 found 392.1577.

1-(adenin-9-yl)-2-deoxy-3-O-(diisopropylphosphonomethyl)-L-threose (19)

To a solution of phenyl(chloro)thiocarbonate (0.25 mL, 1.8 mmol) and DMAP (426 mg, 3.5 mmol) in dried MeCN (25 mL) was added compound **15** (483 mg, 1.2 mmol) at room temperature. The reaction mixture was stirred for 12 hours. The mixture was concentrated, and the residue was purified by column chromatography (CH_2Cl_2:MeOH /10:1) to give 1-(adenin-9-yl)-2-*O*-phenoxythiocarbonyl-3-*O*-diisopropylphosphonomethyl-L-threose as a colorless oil. To the solution of 1-(adenin-9-yl)-2-*O*-phenoxythiocarbonyl-3-*O*-diisopropylphosphonomethyl-L-threose in dried 50 mL of toluene was added tributytinhydride (339μL, 1.2 mmol) and AIBN (48mg, 0.3 mmol). The reaction mixture was refluxed for 6 hours and concentrated in vacuo. The residue was purified by column chromatography (CH_2Cl_2:MeOH/10:1) to give compound **19** (110 mg, 0.27 mmol) as a colorless oil in 23% yield. ^1H NMR (200 MHz, $CDCl_3$) δ_H 1.27-1.34 (m, 12H, CH_3), 2.54-2.75 (m, 2H, C(2)H_2), 3.62-3.82 (m, 2H, P CH_2), 4.04 (dd, J_1 = 10.3 Hz, J_2 = 4.0 Hz, 1H, C(4')H_a), 4.35 (d, J = 10.3 Hz, C(4')H_b), 4.43-4.48 (m, 1H, C(3')H), 4.62-4.84 (m, 2H, O$\underline{C}$H$(CH_3)_2$), 6.21 (br s, 2H, NH_2), 6.47 (dd, J_1 = 7.2 Hz , J_2 = 2.7 Hz, 1H, C(1')H), 8.31(s, 1H, A-C(2)H), 8.33(s, 1H, A-C(8)H); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 23.89 ($CH(\underline{C}H_3)_3$), 38.05 (C-2'), 64.10 (d, $J_{P,C}$ = 169.4 Hz, PCH_2), 71.31 ($\underline{C}H(CH_3)_3$), 71.46 ($\underline{C}H(CH_3)_3$), 73.68 (C-4'), 80.49 (d, $J_{P,C}$ = 10.7 Hz, C-3'), 83.42 (C-1'), 119.50 (A-C(5)), 136.63 (A-C(8)), 149.73 (A-C(6)), 153.07 (A-C(4)), 155.62 (A-C(2)); Exact mass calcd for $C_{16}H_{27}N_5O_5P_1$ $[M+H]^+$ 400.1750 found 400.1740.

1-(thymin-1-yl)-2-deoxy-3-O-(diisopropylphosphonomethyl)-L-threose (20)

This compound was prepared as described for **19**, using **16** (450 mg, 1.1 mmol) as starting material. Column chromatographic purification (CH_2Cl_2:MeOH=10:1) gave

compound 20 (275 mg, 0.70 mmol) as a colorless oil in 64% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.31(d, 6H, CH_3), 1.34(d, 6H, CH_3), 1.97(d, J = 1.1 Hz , 3H, T- CH_3), 2.16(d, J = 15.0 Hz, 1H, C(2') H_a), 2.46-2.62(m, 1H, C(2') H_a), 3.72(d, J = 9.2 Hz, 2H, P CH_2), 3.84(dd, J_1 = 10.6 Hz, J_2 = 3.7 Hz, 1H, C(4') H_a), 4.29-4.37(m, 2H, C(4') H_b, C(3')H), 4.66-4.84(m, 2H, OC<u>H</u>$(CH_3)_2$), 6.24(dd, J_1 = 8.0 Hz, J_2 = 2.6 Hz, 1H, C(1')H), 7.55(d, J = 1.1 Hz, 1H, T-C(6)H), 8.48(s, 1H, NH); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 12.45 (T-CH3), 23.92 (CH(<u>C</u>H3)3), 38.27 (C-2'), 63.99 (d, J =169.2 Hz, PCH_2),, 71.26($CH(CH_3)_3$, 73.36(C-4'), 80.23 (d, J = 10.5 Hz, C-3'), 84.83 (C-1'), 110.72 (T-C(5)), 136.55 (T-C(6)), 150.57 (T-C(2)), 163.80 (T-C(4)); Exact mass calcd for $C_{16}H_{27}N_2O_7P_1Na_1$ $[M+Na]^+$ 413.1454 found 413.1447;

1-(uracil-1-yl)-2-deoxy-3-O-(diisopropylphosphonomethyl)-L-threose (21)

This compound was prepared as described for **19**, using **17** (1.1 g, 2.8 mmol) as starting material. Column chromatographic purification (CH_2Cl_2:MeOH=40:1) gave compound **21** (500 mg, 1.3 mmol) as a colorless oil in 46% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.29-1.34 (m, 12H, CH_3), 2.21 (d, J = 15.4, 1H, C(2')H_a), 2.44-2.60 (m, 1H, C(2')H_b), 3.69 (d, J = 9.2 Hz, 2H, PCH_2), 3.86 (dd, J_1 = 10.6 Hz, J_2 = 3.3 Hz, 1H, C(4')H_a), 4.30-4.38 (m, 2H, C(4')H_b, C(3')H), 4.65-4.81 (m, 2H, OC<u>H</u>$(CH_3)_2$), 5.74 (d, J = 8.1 Hz, 1H, U-C(5)H), 6.21 (dd, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 1H, C(1')H), 7.71 (d, J = 8.0 Hz, 1H, U-C(6)H), 9.16 (s, 1H, NH); ^{13}C NMR (200 MHz, $CDCl_3$) δ_P 23.98 ($CH(\underline{C}H_3)_3$)), 38.42 (C-2')), 63.86 (d, $J_{P,C}$ = 170.7 Hz, PCH_2), 71.26 (<u>C</u>H$(CH_3)_3$), 71.36 (<u>C</u>H$(CH_3)_3$), 73.94 (C-4'), 80.11 (d, $J_{P,C}$ = 11.2 Hz, C-3'), 85.44 (C-1'), 101.95 (U-C(5)), 140.92 (U-C(6)), 150.63 (U-C(2)), 163.47 (U-C(4)); Exact mass calcd for $C_{15}H_{26}N_2O_7P_1$ $[M+H]^+$ 377.1478 found 377.1479.

1-(cytosin-1-yl)-2-deoxy-3-O-(diisopropylphosphonomethyl)-L-threose (22)

To the solution of 1,2,4-triazole (662 mg, 9.6 mmol) in 15 mL pyridine was added phosphorousoxychloride (223 µL, 2.4 mmol) at room temperature. The mixture was stirred for 10 min.. Then the solution of **21** (289 mg, 0.80 mmol) was added to the mixture. The reaction mixture was stirred for 4 hours. Then ammonia gas was bubbled in to the reaction mixture for 1-3 hours and the reaction mixture was concentrated in vacuo.

The residue was purified by column chromatography (CH_2Cl_2:MeOH=12:1) to give compound **22** (220 mg, 0.58 mmol) as a colorless foam in 73% yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.22-1.30 (m, 12H, CH_3), 2.27 (d, J = 15.0, 1H, C(2')H_a), 2.41-2.55 (m, 1H, C(2')H_b), 3.63 (d, J = 9.5 Hz, 2H, PCH_2), 3.91 (dd, J_1 = 10.3, J_2 = 3.5, 1H, C(4')H_a), 4.22-4.36 (m, 2H, C(4')H_b, C(3')H), 4.56-4.76 (m, 2H, OC<u>H</u>$(CH_3)_2$), 5.77 (d, J = 7.3 Hz, 1H, C-C(5)H),6.17 (dd, J_1 = 7.3, J_2 = 1.8, 1H, C(1')H), 7.67 (d, J = 7.3 Hz, 1H, C-C(6)H), 8.18 (s, 2H, NH_2); ^{13}C NMR (200 MHz, $CDCl_3$) δ_C 23.80 ($CH(\underline{C}H_3)_3$), 38.46 (C-2'), 63.66 (d, $J_{P,C}$ = 172.2 Hz, PCH_2), 71.48 ($\underline{C}H(CH_3)_3$), 71.60 ($\underline{C}H(CH_3)_3$), 71.75 ($\underline{C}H(CH_3)_3$), 74.12 (C-4'), 80.40 (d, $J_{P,C}$ = 11.2 Hz, C-3'), 86.68 (C-1'), 94.21 (C-C(5)), 141.9 (C-C(6)), 156.58 (C-C(2)), 165.83 (C-C(1)); Exact mass calcd for $C_{15}H_{26}N_3O_6P_1Na_1$ $[M+H]^+$ 376.1637 found 376.1638.

1-(adenin-9-yl)-3-O-(phosphonomethyl)-L-threose sodium salt (3a)

To a solution of **15** (220 mg, 0.55 mmol) and Et_3N (1mL) in DCM (9 mL) was added bromotrimethylsilane (290 µL, 2.2 mmol) at room temperature. The reaction mixture was stirred for 48 hours. The reaction was quenched with 1.0 M TEAB solution. The mixture was concentrated, and the residue was purified by column chromatography (CH_2Cl_2:MeOH /2:1, 1:1, 1:2) to give crude title compound. Purification using sephadex-DEAE A-25 with gradient TEAB solution from 0.01 M to 0.5 M and ion exchanges by the Dowex-Na+ resin offered **3a** (96 mg, 0.25 mmol) as a colorless solid in 45% yield. ^{1}H NMR (500MHz, D_2O) δ_H 3.54-3.62 (m, 2H, PCH_2), 4.32-4.39 (m, 3H, C(4')H_2, C(3')H), 4.82 (dd, J_1 = 2.4 Hz, J_2 = 2.0 Hz, 1H, C(2')H), 6.09 (d, J = 2.4 Hz, 1H, C(1')H), 8.23 (s, 1H, A-C(8)H), 8.45 (s, 1H, A-C(2)H); ^{13}C NMR (500MHz, D_2O): δ_c 70.1(d, $J_{P,C}$ = 164.6 Hz, PCH_2), 75.38 (C-4'), 80.70 (C-2'), 87.56 ($J_{P,C}$ = 9.8 Hz, C-3'), 91.93 (C-1'), 121.21 (A-C(5), 143.74 (A-C(8)), 151.49 (A-C(6), 155.48 (A-C(4), 158.30 (A-C(2)); ^{31}P NMR (500MHz, D_2O): δ_p 13.64; Exact mass calcd for $C_{10}H_{13}N_5O_6P_1$ $[M-H]^-$ 330.0603 found 330.0602.

1-(thymin-1-yl)-3-O-(phosphonomethyl)-L-threose sodium salt (3b)

This compound was prepared as described for **3a**, using **16** (220 mg, 0.58 mmol) as starting material. Compound **3b** (90 mg, 0.24 mmol) was obtained as a colorless solid in

42% yield. ^{1}H NMR (500MHz, D_2O) δ_H 1.89 (s, 3H, T-CH_3), 3.60-3.68 (m, 2H, PCH_2), 4.16 (d, J = 4.1 Hz, 1H, C(3')H), 4.24(dd, J_1 = 10.7 Hz, J_2 = 4.1 Hz, 1H, C(4')H_a), 4.42 (d, J = 10.7 Hz, 1H, C(4')H_b), 4.45 (s, 1H, C(2')H), 5.85 (d, J = 1.2 Hz,1H, C(1')H), 7.59-7.60 (m, 1H, T-C(6)H); ^{13}C NMR (500MHz, D_2O): δ_C 14.28 (T-CH3), 67.95 (d, $J_{P,C}$ = 157.2 Hz, PCH_2), 75.78 (C-4'), 80.17 (C-2'), 87.27 (d, $J_{P,C}$ = 11.7 Hz, C-3'), 94.22 (C-1'), 113.36 (T-C(5)), 140.66 (T-C(6)), 154.30 (T-C(2)), 169.39 (T-C(4)); ^{31}P NMR (500MHz, D_2O) δ_P 15.68; Exact mass calcd for $C_{10}H_{14}N_2O_8P_1$ $[M-H]^-$ 321.0488 found 321.0474.

1-(uracil-1-yl)-3-O-(phosphonomethyl)-L-threose sodium salt (3c)

This compound was prepared as described for **3a** using **17** (200 mg, 0.53 mmol) as starting material and TBMSBr (200 mL, 2.1 mmol). Compound **3c** (93 mg, 0.26 mmol) was obtained as a colorless solid in 49% yield. ^{1}H NMR (500 MHz, D_2O) δ_H 3.58-3.67 (m, 2H, PCH_2), 4.16 (d, J = 3.3 Hz, 1H, C(3')H), 4.26 (dd, J_1 = 10.7 Hz, J_2 = 3.9 Hz, 1H, C(4')H_a), 4.45 (d, J = 10.7 Hz, 1H, C(4')H_b), 4.47 (s, 1H, C(2')H), 5.85 (d, J = 8.0 Hz, 1H, U-C(5)H), 5.85 (s, 1H, C(1')H), 7.80 (d, J = 8.1 Hz, 1H, U-C(6)H); ^{13}C NMR (500 MHz, D_2O) δ_C 67.98 (d, J= 156.2 Hz, PCH_2), 76.22 (C-4'), 80.09 (C-2'), 87.15 (d, J= 11.7 Hz, C-3'), 94.63 (C-1'), 104.09 (U-C(5)), 145.23 (U-C(6)), 154.26 (U-C(2)), 169.22 (U-C(4)); ^{31}P NMR (500 MHz, D_2O) δ 15.37; Exact mass calcd for $C_9H_{12}N_2O_8P_1$ $[M-H]^-$ 307.0331 found 307.0325.

1-(cytosin-1-yl)-3-O-(phosphonomethyl)-L-threose sodium salt (3d)

This compound was prepared as described for **3a**, using **18** (150 mg, 0.38 mmol) as starting material. Compound **3d** (58 mg, 0.16 mmol) was obtained as a colorless solid in 43% yield. ^{1}H NMR (500MHz, D_2O) δ_H 3.53-3.62 (m, 2H, PCH_2), 4.15 (d, J = 3.7 Hz, 1H, C(3')H), 4.27 (dd, J_1 = 10.7 Hz, J_2 = 3.7 Hz, 1H, C(4')H_a), 4.42 (s, 1H, C(2')H), 4.44 (d, J = 10.7 Hz, 1H, C(4')H_b), 5.86 (s, 1H, C(1')H), 6.01 (d, J = 7.6 Hz, C-C(5)H), 7.77 (d, J = 7.6 Hz, C-C(6)H); ^{13}C NMR (500MHz, D_2O) δ_C 68.0 (d, $J_{P,C}$ = 156.2 Hz, PCH_2), 76.17 (C-4')), 80.13 (C-2')), 87.27 (d, $J_{P,C}$ = 11.8 Hz, C-3')), 95.16 (C-1'), 98.23(C-C(5)), 145.04(C-C(6)), 160.06(C-C(2)), 168.84(C-C(4)); ^{31}P NMR (500MHz, D_2O) δ_P 15.28; Exact mass calcd for $C_9H_{13}N_3O_7P_1$ $[M-H]^-$ 306.0491 found 306.0481.

1-(adenin-1-yl)-2-deoxy-3-O-(phosphonomethyl)-L-threose sodium salt (3e)

This compound was prepared as described for **3a**, using **19** (70 mg, 0.23 mmol) as starting material. Compound **3e** (38 mg, 0.11 mmol) was obtained as a colorless solid in 43% yield. ^{1}H NMR (500MHz, D_2O, 60°C) δ_H 2.63(dd, J_1 = 15.5 Hz, J_2 = 1.3 Hz, 1H, C(2')H_a), 2.75-2.81 (m, 1H, C(2')H_b), 3.55-3.64 (m, 2H, P CH_2), 4.09 (dd, J_1 = 10.0 Hz, J_2 = 4.0 Hz, 1H, C(4')H_a), 4.33 (d, J = 10.0 Hz, 1H, C(4')H_b), 4.51 (dd, J_1 = 5.5 Hz, J_2 = 4.5 Hz, 1H, C(3')H), 6.39 (dd, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 1H, C(1')H), 8.22 (s, 1H, C(2)H), 8.49 (s, 1H, C(8)H); ^{13}C NMR (500MHz, D_2O) δ_C 39.78(C-2'), 68.34 (d, $J_{P,C}$ = 155.2 Hz, PCH_2), 76.53 (C-4'), 82.79 (d, $J_{P,C}$ = 11.9 Hz, C-3'), 86.32 (C-1'), 121.13 (A-C(5)), 143.89 (A-C(8)), 151.33 (A-C(6)), 155.25 (A-C(4)), 158.18 (A-C(2)); ^{31}P NMR (500MHz, D_2O) δ_P 154.46; Exact mass calcd for $C_{10}H_{13}N_5O_5P_1$ $[M-H]^-$ 314.0654 found 314.0632.

1-(thymin-1-yl)-2-deoxy-3-O-(phosphonomethyl)-L-threose sodium salt (3f)

To a solution of **20** (260 mg, 0.67 mmol) and Et_3N (1 mL) in DCM (25 mL) was added iodotrimethysilane (0.73 mL, 5.36 mmol) at 0C. The reaction mixture was stirred for 2 hours. The reaction was quenched with 1.0 M TEAB solution. The mixture was concentrated, and the residue was purified by column chromatography (CH_2Cl_2:MeOH /2:1, 1:1, 1:2) to give crude **3f**. Purification using sephadex-DEAE A-25 with gradient TEAB solution from 0.01 M to 0.5 M and ion exchanges by the Dowex-Na^+ resin offered **3f** (95 mg, 0.27 mmol) as a colorless solid in 40% yield. ^{1}H NMR (500 MHz, D_2O) δ_H 1.91(s, 3H, T-CH_3), 2.29(d, J = 15.4 Hz, 1H, C(2')H_a), 2.58-2.64 (m, 1H, C(2')H_a), 3.57-3.65 (m, 2H, PCH_2), 3.95(dd, J_1 = 10.5 Hz, J_2 = 3.4 Hz, 1H, C(4')H_a), 4.38-4.41 (m, 2H, C(4')H_b, C(3')H), 6.20(dd, J_1 = 8.3 Hz, J_2 = 2.4 Hz, 1H, C(1')H), 7.78(d, J = 1.0 Hz, 1H, T-C(6)H); ^{13}C NMR (500 MHz, D_2O) δ_C 14.50(T-CH_3), 39.62(C-2'), 67.81 (d, J = 158.1 Hz, PCH_2), 76.63 (C-4'), 82.66 (d, J = 11.3 Hz, C-3'), 88.41 (C-1'), 113.94 (T-(C(5)), 141.32 (T-(C(6)), 154.68 (T-(C(2)), 169.51 (T-C(4)); ^{31}P NMR (500 MHz, D_2O) δ_P 16.02; Exact mass calcd for $C_{10}H_{14}N_2O_7P_1$ $[M-H]^-$ 305.0538 found 305.0537.

1-(uracil-1-yl)-2-deoxy-3-O-(phosphonomethyl)-L-threose sodium salt (3g)

This compound was prepared as described for **3f**, using **21** (154 mg, 0.41 mmol) as starting material and iodotrimethysilane (0.47 mL, 3.3 mmol). Compound **3g** (50 mg, 0.14 mmol) was obtained as a colorless solid in 34% yield. ^{1}H NMR (500 MHz, D_2O) δ_H 2.31-2.35 (m, 1H, C(2')H_a), 2.57-2.62 (m, 1H, C(2')H_b), 3.54-3.62 (m, 2H, PCH_2), 3.97 (dd, J_1 = 10.5 Hz, J_2 = 3.7 Hz, 1H, C(4')H_a), 4.38-4.40 (m, 1H, C(3')H), 4.42 (dd, J_1 = 10.5 Hz, J_2 = 2.0 Hz, 1H, C(4')H_b), 5.88 (d, J = 8.3 Hz, 1H, U-C(5)H), 6.21 (dd, J_1 = 8.2 Hz, J_2 = 2.0 Hz, 1H, C(1')H), 7.99 (d, J = 8.2Hz, 1H, U-C(6)H); ^{13}C NMR (500 MHz, D_2O) δ_C 39.46 (C-2')), 67.56 (d, J = 156.9 Hz, PCH_2), 76.77 (C-4'), 82.31 (d, J = 13.8 Hz, C-3'), 88.57 (C-1'), 101.45 (U-C(5)), 145.81 (U-C(6), 169.23 (U-C(4)); ^{31}P NMR (500 MHz, D_2O) δ_P 15.72; Exact mass calcd for $C_9H_{12}N_2O_7P_1$ $[M-H]^-$ 291.0382 found 291.0391.

1-(cytosin-1-yl)-2-deoxy-3-O-(phosphonomethyl)-L-threose sodium salt (3h)

This compound was prepared as described for **3f**, using **22** (200 mg, 0.53 mmol) as starting material and iodotrimethysilane (0.6 mL, 4.2 mmol). Compound **3h** (130 mg, 0.38 mmol) was obtained as a colorless solid in 73 % yield. ^{1}H NMR (500 MHz, D_2O) δ_H 2.32 (d, J = 15.3, 1H, C(2')H_a), 2.56-2.61 (m, 1H, C(2')H_b), 3.52-3.61 (m, 2H, PCH_2), 4.01 (dd, J_1 = 10.5 Hz, J_2 = 3.6 Hz, 1H, C(4')H_a), 4.39-4.40 (m, 1H, C(3')H), 4.44 (dd, J1=10.7, J2= 1.7 Hz, C(4')H_b), 6.06 (d, J = 7.6 Hz, 1H, C-C(5)H),6.20 (dd, J_1 = 7.8 Hz, J_2 = 2.0 Hz ,1H, C(1')H), 7.95 (d, J = 7.6 Hz, 1H, C-C(6)H); ^{13}C NMR (500 MHz, D_2O) δ_C 37.22 (C-2'), 64.88 (d, J = 157.2 Hz, PCH_2), 74.37 (C-4'), 79.99 (d, J = 11.7 Hz, C-3'), 86.71 (C-1'), 95.99 (C-C(5)), 143.02 (C-C(6), 157.56 (C-C(2)), 169.2 3(C-C(4)); ^{31}P NMR (500 MHz, D_2O) δ_p 15.96; Exact mass calcd for $C_9H_{13}N_3O_6P_1$ $[M-H]^-$ 290.0535 found 290.0542.

Example 5: Antiviral activity

Compounds 3a-h were evaluated for their potential to inhibit the replication of HIV in a cell culture model for acute infection. The cytotoxicity of the compounds was determined in parallel. The origin of the HIV-1 (III_B) virus stock[18] and the HIV-2 (ROD)[19] stock has been described. They were obtained from the culture supernatant of HIV-1 or HIV-2 infected MT-4 cells, respectively. . The inhibitory effect of the

compounds on HIV-1 and HIV-2 replication were monitored by measuring the viability of MT-4 cells 5 days after infection.[20] Cytotoxicity of the compounds was determined in parallel by measuring the viability of mock-infected cells on day 5, using a tetrazolium based colorimetric method to determine the number of viable cells.

PMDTA shows an IC_{50} value of 1.0 μg/mL both against HIV-1 and HIV-2. PMDTT has an IC_{50} value of 2.4 μg/mL against HIV-1 and HIV-2. No cytotoxicity was observed for PMDTA nor PMDTT at the highest concentration tested (125 μg/mL), giving the compounds a SI of > 125 (PMDTA) and > 50 (PMDTT) in these cellular systems. In the cellular test system, both compounds are as active as PMEA and PMPA, and their cytotoxicity is lower.[11]

REFERENCES TO THIS APPLICATION

1. Parikh, J.R., Wolff, M.E., Burger, A. Analogs of nucleotides. II. Phosphonate esters of ribose and glucopyranosyl purine derivatives. *J.Am.Chem.Soc.*, 79: 2778-2781, 1957
2. Wolff, M.E., Burger, A., Analogs of nucleotides. III. Synthesis in the series of adenosine phosphonate derivatives, *J.Am.Pharm.Ass.*, 48: 56-59, 1959
3. Yengoyan, L., Rammler, D.H. Nucleoside phosphonic acids. I. The synthesis of 5'-deoxyuridine 5'-phosphonic acids and derivatives. *Biochemistry*, 5: 3629-3638, 1966
4. Rammler, D.H., Yengoyan, L., Paul, A.V., Bax, P.C. Nucleoside phosphonic acids. II. The synthesis of 5'-deoxythymidine 5'-phosphonic acid and its pyrophosphate derivatives. *Biochemistry*, 6: 1828-1837, 1967
5. Holý, A. Synthesis of 5'-deoxyuridine 5'-phosphonic acid. *Tetrahedron Lett.*, 10: 881-884, 1967
6. Jones, G.H., Moffatt, J.G. The synthesis of 6'-deoxyhomonucleoside-6'-phosphonic acids. *J.Am.Chem.Soc.*, 90: 5337-5338, 1968
7. Holý, A., Votruba, I., Merta, A. Cerny, J., Vesely, J., Vlach, J., Sediva, K., Rosenberg, I., Otmar, M., Hrebabecky, H., et al. Acyclic nucleotide analogues: synthesis, antiviral activity and inhibitory effects on some cellular and virus-encoded enzymes in vitro. *Antiviral Res.*, 13: 295-311, 1990
8. for example:
   Montgomery, J.A., Hewson, K., The synthesis of phosphonic acid analogues of purine ribonucleotides: an exception to the trans rule. *Chem. Commun.*, 15-16, 1969
   Hampton, A., Perini, F., Harper, P.-J. Interactions of the epimeric 5'-C-methyl and 5'-C-carbamyl derivatives of adenosine monophosphate with adenosine monophosphate utilizing enzymes. *Biochemistry*, 12: 1730-1736, 1973
   Fuertes, M., Witkowski, J.T., Streeter, D.G., Robins, R.K. Synthesis and enzymatic activity of 1,2,4-triazole-3-carboxamide 6'-deoxyhomoribonucleoside-6'-phosphonic acid and related compounds. *J. Med. Chem.*, 17: 642-645, 1974

Holý, A., Rosenberg, I. Preparation of 5'-*O*-Phosphonylmethyl analogues of nucleoside-5'-phosphates, 5'-diphosphates and 5'-triphosphates, *Collect. Czech Chem. Commun.*, **47**: 3447-3463, 1982

Morr, M., Ernst, L., Grotjahn, L. 2',3'-Dideoxy-3'-*C*-(phosphono-methyl)adenosine, the phosphonate analogue of 2'-deoxyadenosine 3'-phosphate. *Z. Naturforsch.*, **38B**: 1665-1668 ,1983

Hollmann, H., Schlimme, E. *Liebigs Annal.* Darstellung und konformationszuordnung einiger 5-homologer adenosinederivate, 98-107, 1984

Barton, D.H.R., Géro, S.D., Quiclet-Sire, B., Samadi, M. Radical addition to vinyl phosphonates. A new synthesis of isosteric phosphonates and phosphonate analogues of α-amino acids. *J. Chem. Soc. Chem. Commun.* 1000-1001, 1989

Liu, J., Van Aerschot, A., Balzarini, J., Janssen, G., Busson, R., Hoogmartens, J., De Clercq, E., Herdewijn, P. 5'-*O*-phosphonomethyl-2',3'-dideoxynucleosides: synthesis and anti-HIV activity *J. Med. Chem.*, **33**: 2481-2487, 1990

9. Pérez-Pérez, M.J., Rozenski, J., Herdewijn, P., Stereospecific synthesis of a pentopyranosyl analogue of D4T monophosphate, *Bioorg. Med. Chem. Lett.*, **4**: 1199-1202, 1994

Pérez-Pérez, M.-J., Doboszewski, B., Rozenski, J., Herdewijn, P., Stereocontrolled synthesis of phosphonate derivatives of tetrahydro- and dihydro-2*H*-pyranyl nucleosides: the selectivity of the Ferrier rearrangement *Tetrahedron Assym.*, **6**: 973-984, 1995

10. for example: Wolff-Kugel, D., Halazy, S. *Tetrahedron Lett.*, **32**: 6341-6344, 1991

Bronson, J.J., Ferrara, L.M., Martin, J.C., Synthesis of new carbocyclic phosphonate analogs of dideoxypurine nucleotides ,*Bioorg. Med. Chem. Lett.*, **2**, 685-690, 1992

Jähne, G., Müller,A., Kroha, H., Rösner, M., Holzhäuser, O., Meichsner, C., Helsberg, M., Winkler, I., Rieß, G., Preparation of carbocyclic phosphonate nucleosides, *Tetrahedron Lett.*, **33**: 5335-5338, 1992

Coe, D.M., Garofalo, A., Roberts, S.M., Storer, R., Thorpe, A.J., Synthesis of the phosphonate isostere of carbocyclic 5- bromovinyldeoxyuridine monophosphate, *J. Chem. Soc., Perkin I*, 3061-3063, 1994

Pérez-Pérez, M.J., Rozenski, J., Busson, R., Herdewijn, P., Application of the

Mitsunobu-type condensation reaction to the synthesis of phosphonate derivatives of cyclohexenyl and cyclohexanyl nucleosides „*J. Org. Chem.*, **60**: 1531-1537, 1995

Liboska, R., Masojidkova, M., Rosenberg, I., Carbocyclic phosphonate-based nucleotide analogs related to PMEA. I. Racemic trans-configured derivatives, *Coll. Czech. Chem. Commun.*, **61**: 313-332, 1996

11. For a review on chemistry and biology of isopolar phosphorus-modified nucleotide analogues:

Holý, A., Isopolar Phosphorus-Modified Nucleotide Analogues, *in Advances in Antiviral Drug Design*, E. De Clercq, ed., vol. 1, 179-231, 1993

12. Lee, H., Hanes, J., Johnson, K.A., Toxicity of nucleoside analogues used to treat AIDS and the selectivity of the mitochondrial DNA polymerase, *Biochemistry*, **42**, 14711-14719, 2003

1. Schöning, K.-U., Scholz, P., Guntha, S., WU, X., Krishnamurthy, R., Eschenmoser, A., Chemical etiology of nucleic acid structure: the □-threofuranosyl-(3'> 2') oligonucleotide system, *Science*, **290**: 1347-1351, 2000

14. Kempeneers, V., Vastmans, K., Rozenski, J., Herdewijn, P., Recognition of threosyl nucleotides by DNA and RNA polymerases, *Nucleic Acids Res.*, **31**: 6221-6226, 2003

15. Chaput, J.C., Szostack, J.W., TNA synthesis by DNA polymerase, *J. Am. Chem. Soc.*, **125**: 9274-9275, 2003

16. Barton, D.H., Subramanian, R., Reactions of relevance to the chemistry of aminoglyxoside antibiotics. Part 7. Conversion of thiocarbonates into deoxysugars, *Chem. Soc. Perkin Trans. I*, 15: 1718-1723, 1977

17. Barton, D.H., McCombie, S.W., A new method for the deoxygenation of secondary alcohols, *J. Chem. Soc. Perkin Trans. I*, 1574-1585, 1975.

18. Popovic, M., Sarngadharan, M.G., Read, E., Gallo, R.C., Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS, *Science*, **224**: 497-500, 1984.

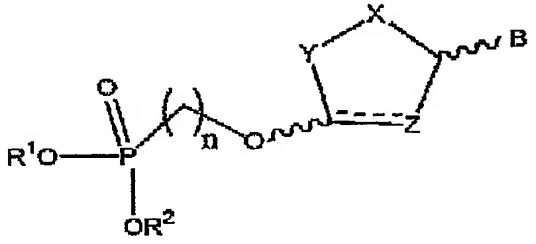
19. Barre-Sinoussi, F., Chermann, J.C., Rey, F., Nugeyre, M.T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., Rozenbaum, W.,

Montagnier, L., Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS), *Science*, **220**: 868-871, 1983.

20. Pauwels, R., Balzarini, J., Baba, M., Snoeck, R., Schols, D., Herdewijn, P., Desmyter, J., De Clercq, E., Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds *J. Virol. Methods*, **20**: 309-321, 1988.

CLAIMS

1. A phosphonoalkoxy substituted nucleoside, comprising a five-membered, saturated or unsaturated, oxygen, nitrogen or sulfur containing ring or analogues or derivatives thereof corresponding to the formula I, isomers, solvates or pharmaceutical salts thereof,



(I)

wherein:

- B is a heterocycle selected from the group consisting of pyrimidine and purine bases;
- The dotted line represents an optional double bond, provided that if Z is oxygen, or sulfur, there is no double bond;
- Each R^1 and R^2 are independently selected from the group of hydrogen; $(-PO_3R^6)_m$-$PO_3R^7R^8$; alkyl; alkenyl; alkynyl; cycloalkyl; cycloalkenyl; cycloalkynyl; aryl; arylalkyl; heterocyclic ring; heterocyclic ring-alkyl; acyloxyalkyl; acyloxyalkenyl; acyloxyalkynyl; acyloxyaryl; acyloxyarylalkyl; acyloxyarylalkenyl; acyloxyarylalkynyl; dialkylcarbonate; alkylarylcarbonate; alkylalkenylcarbonate; alkylalkynylcarbonate; alkenylarylcarbonate; alkynylarylcarbonate; alkenylalkynylcarbonate; dialkenylcarbonate; dialkynylcarbonate; wherein said alkyl, alkenyl and alkynyl can contain a heteroatom in or at the end of the hydrocarbon chain, said heteroatom selected from O, S and N;
- Each X, Y and Z are independently selected from the group of oxygen; nitrogen; sulfur; CHR^3; wherein at least one of X, Y or Z is oxygen, nitrogen or sulfur and maximally two of X, Y or Z are oxygen, nitrogen or sulfur and the other(s) is/are CHR^3;
- R^3 is selected from hydrogen; azido; F and OR^4;

- R^4 is selected from hydrogen; alkyl; alkenyl; alkynyl; cycloalkyl; cycloalkenyl; cycloalkynyl; aryl; heterocyclic ring; arylalkyl; heterocyclic ring-alkyl; acyloxyalkyl; wherein said alkyl, alkenyl and alkynyl can contain a heteroatom in or at the end of the hydrocarbon chain, said heteroatom selected from O, S and N;
- Each R^6, R^7 and R^8 are independently selected from hydrogen; alkyl; alkenyl; alkynyl; cycloalkyl; cycloalkenyl; cycloalkynyl; aryl; arylalkyl; heterocyclic ring; heterocyclic ring-alkyl; acyloxyalkyl; wherein said alkyl, alkenyl and alkynyl can contain a heteroatom in or at the end of the hydrocarbon chain, said heteroatom selected from O, S and N;
- n is selected from 1 to 6;
- m is 0 or 1.

2. A phosphonoalkoxy substituted nucleoside according to claim 1, wherein each X, Y and Z are independently selected from the group of oxygen; nitrogen; sulfur; CHR^3; wherein at least one of X, Y or Z is oxygen, nitrogen or sulfur and maximally two of X, Y or Z are oxygen, nitrogen or sulfur and the other(s) is/are CHR^3;

3. The phosphonoalkoxy substituted nucleoside according to claim 1 or 2, wherein said phosphonoalkoxy substituted nucleoside is a 3'-phosphonalkoxy substituted furanose nucleoside.

4. The phosphonoalkoxy substituted nucleoside according to claim 1 to 3, wherein there is no double bond in the five-membered ring, X is oxygen and Y and Z are CHR^3.

5. The phosphonoalkoxy substituted nucleoside according to any of claims 1 to 4, being selected from the following group:
1-(N^6-benzoyladenin-9-yl)-2-O-benzoyl-3-O-(diisopropylphosphonomethyl)-L-threose (**11**);
1-(thymin-1-yl)-2-O-benzoyl-3-O-(diisopropylphosphonomethyl)-L-threose (**12**);
1-(uracil-1-yl)-2-O-benzoyl-3-O-(diisopropylphosphonomethyl)-L-threose (**13**);

1-(N^4-acetylcytosin-1-yl)-2-O-benzoyl-3-O-(diisopropylphosphonomethyl)-L-threose (**14**);

1-(adenin-9-yl)-3-O-(diisopropylphosphonomethyl)-L-threose (**15**);

1-(thymin-1-yl)-3-O-(diisopropylphosphonomethyl)-L-threose (**16**);

1-(uracil-1-yl)-3-O-(diisopropylphosphonomethyl)-L-threose (**17**);

1-(cytosin-1-yl)-3-O-(diisopropylphosphonomethyl)-L-threose (**18**);

1-(adenin-9-yl)-2-deoxy-3-O-(diisopropylphosphonomethyl)-L-threose (**19**);

1-(thymin-1-yl)-2-deoxy-3-O-(diisopropylphosphonomethyl)-L-threose (**20**);

1-(uracil-1-yl)-2-deoxy-3-O-(diisopropylphosphonomethyl)-L-threose (**21**);

1-(cytosin-1-yl)-2-deoxy-3-O-(diisopropylphosphonomethyl)-L-threose (**22**);

1-(adenin-9-yl)-3-O-(phosphonomethyl)-L-threose sodium salt (**3a**);

1-(thymin-1-yl)-3-O-(phosphonomethyl)-L-threose sodium salt (**3b**);

1-(uracil-1-yl)-3-O-(phosphonomethyl)-L-threose sodium salt (**3c**);

1-(cytosin-1-yl)-3-O-(phosphonomethyl)-L-threose sodium salt (**3d**);

1-(adenin-1-yl)-2-deoxy-3-O-(phosphonomethyl)-L-threose sodium salt (**3e**);

1-(thymin-1-yl)-2-deoxy-3-O-(phosphonomethyl)-L-threose sodium salt (**3f**);

1-(uracil-1-yl)-2-deoxy-3-O-(phosphonomethyl)-L-threose sodium salt (**3g**);

1-(cytidin-1-yl)-2-deoxy-3-O-(phosphonomethyl)-L-threose sodium salt (**3h**);

a pharmaceutically acceptable salt , an isomer or a solvate thereof.

6. A process for preparing a phosphonoalkoxy substituted nucleoside, an isomer, a pharmaceutically acceptable salt or a solvate thereof according to any of the claims 1 to 5, said process comprising the steps of reacting a protected oxygen containing five-membered ring with protected phosphonylalkyl, followed by reaction with a pyrimidine or purine base, deprotection of the five-membered ring protecting groups and purine or pyrimidine base protecting groups, if necessary a deoxygenation step and finally a deprotection of the phosphonate protecting groups.

7. The use of a phosphonoalkoxy substituted nucleoside according to any of the claims 1 to 5, for the manufacture of a medicine.

8. The use according to claim 7, wherein said medicine is for the prevention or treatment of viral infections in a mammal.

9. The use according to claim 8, wherein said viral infection is an infection by the Human Immunodeficiency Virus (HIV).

10. A pharmaceutical composition comprising phosphonoalkoxy substituted nucleoside according to any of the claims 1 to 5 as an active ingredient in admixture with at least a pharmaceutically acceptable carrier.

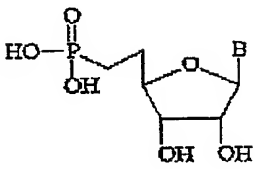
11. A pharmaceutical composition according to claim 10, having antiviral activity.

12. A method of treatment or prevention of a viral infection in a mammal, comprising administering to the mammal in need of such treatment a therapeutically effective amount of a phosphonoalkoxy substituted nucleoside according to any of the claims 1 to 5.

ABSTRACT

The present invention relates to novel phosponate nucleosides, more specifically to novel phosponalkoxy substituted nucleosides. The invention further relates to compounds having HIV (Human Immunodeficiency Virus) replication inhibiting properties and to compounds having antiviral activities with respect to other viruses. The invention also relates to methods for preparation of all such compounds and pharmaceutical compositions comprising them. The invention further relates to the use of said compounds as a medicine and in the manufacture of a medicament useful for the treatment of subjects suffering from HIV infection, as well as for treatment of other viral, retroviral or lentiviral infections and to the treatment of animals suffering from FIV, viral, retroviral or lentiviral infections. The invention also provides a method of treatment or prevention of a viral infection in a mammal, comprising administering to the mammal in need of such treatment a therapeutically effective amount of a phosphonoalkoxy substituted nucleoside.

Figures



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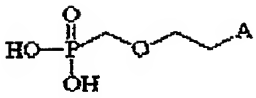

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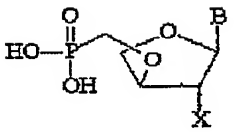
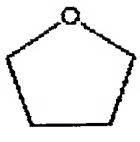
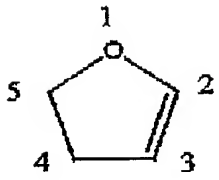


3 a X = OH, B = adenin-1-yl
b X = OH, B = thymin-1-yl
c X = OH, B = uracil-1-yl
d X = OH, B = cytosin-1-yl
e X = H, B = adenin-1-yl (PMDTA)
f X = H, B = thymin-1-yl (PMDTT)
g X = H, B = uracil-1-yl
h X = H, B = cytosin-1-yl

Figure 1



Tetrahydro-furan



2,3-Dihydro-furan

Furan

Figure 2

13 -04- 2005